JOURNAL OF AGRICULTURAL RESEARCH

VOL. 38 WASHINGTON, D. C., JUNE 15, 1929 No. 12

PATHOLOGICAL SYMPTOMS IN LEGUMES CAUSED BY THE POTATO LEAF HOPPER[1]

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INTRODUCTION

For several years it has been recognized that the potato leaf hopper (*Empoasca fabae* Harris) causes serious damage to potatoes and that the same insect, earlier described as apple leaf hopper (*Empoasca mali* Le B.), may be an important pest of apples, beans, and other crops. For years it was assumed that the injury produced by the leaf hopper was merely a mechanical one resulting from puncture of the plant tissue and extraction of food materials, similar to that occurring during a heavy infestation of aphids or other sucking insects. The observations reported by Ball (*1*),[3] and by others since the time of his publication, have indicated, however, that the burning produced by the potato leaf hopper must be due to something more than punctures and depletion of plant food or water.

The phases of the problem involving studies of the plant in its response to attacks by this insect, of the pathological symptoms on various hosts, of the actual cause of the disturbance within the plant, of the varietal differences in degree of susceptibility, and the like have been little studied since Ball definitely attributed tipburn or "hopper burn" to this species of leaf hopper. The work here reported in no sense solves any of these problems. Its purpose is chiefly to call attention to the damage caused by this insect to an important group of cultivated plants, to pathological conditions other than tip and marginal burning, and to indicate further the lack of knowledge as to the nature of the injury to plants caused during or following the feeding of *Empoasca fabae*.

The injuries to clover associated with leaf hoppers have been observed practically all through the clover belt of this country. The yellow top or "yellows" of alfalfa is also common in the same area. No information is available as to how serious and widespread the leaf-hopper injury to forage crops may be in the western section of this country and in places outside the boundaries of the United States. In the far Western States these injuries are apparently not considered serious, if they occur. The fact that the most susceptible clovers, even Ladino clovers, flourish in portions of the Northwestern States might be interpreted as indicating that there the insect is not a serious factor in clover culture, although it has been found in that region.

[1] Received for publication Oct. 6, 1928, issued June, 1929.

[2] The experimental work here reported was conducted independently at the Arlington Experiment Farm, Rosslyn, Va., by the senior writer, and at the Illinois Agricultural Experiment Station by the junior writer. The writers are indebted to A. J. Pieters for aid in conducting the work and in preparing the manuscript.

[3] Reference is made by number (italic) to "Literature cited," p. 676.

HOST PLANTS

That this species of leaf hopper, reported under several different names, might attack a great variety of plants has long been recognized by entomologists. It has been reported most frequently as a pest of potatoes and apples, but since reviews and lists of the printed records are elsewhere available it is unnecessary in this report to make extensive reference to those works.

The potato leaf hopper was reported as a pest of legumes years ago. In 1900, Forbes and Hart (*6*) reported it on a number of hosts, Harris's type specimen was recorded on beans (*2*, *12*), Gossard (*9*) reported it on clover, and Garman (*7*), Washburn (*18*), and Webster (*19*) found it on red clover, alfalfa, sweet clover, and other legumes. Osborn (*14*) in 1912 reported it as causing injury to soy beans, alfalfa, cowpeas, and clover. He recorded an instance where it was found to be abundant on soy beans and "the leaves were corrugated and curled by them. The lower leaves are yellowish." He further cited a report from Ohio: "It attacked second crop of alfalfa and it is turning yellow from the effect." Referring to alfalfa, he stated that "the species must be reckoned with in the growing of this crop." In 1914 Piper (*15*) noted the association of leaf hoppers with alfalfa yellows. Others since that time have reported this leaf hopper as feeding on forage crops and in some cases have noted that injury resulted.

Until recently little or nothing had been done to determine under controlled conditions the actual injury to this group of plants that could be definitely attributed to this insect. During the summer of 1926 the senior writer was called upon to determine the cause of a "diseased condition" prevailing on red-clover plants grown in pot cultures at Urbana, Ill. The browning of the leaves and the abundance of the potato leaf hopper at once suggested hopper burn, and the reddening and yellowing of the leaves coincident with this burning seemed likely to be due to the same cause. Experiments were then outlined which when completed showed that this diagnosis was correct (*13*). Granovsky (*11*) the same year determined by cage experiments that this species of leaf hopper caused the yellow top of alfalfa.

METHODS

Field observations during the summers of 1926 and 1927 indicated that several forage-crop legumes, as well as the garden bean (*Phaseolus vulgaris*), were suffering to a marked degree from attacks of the potato leaf hopper. During the summer of 1927 further experimental work was conducted to determine somewhat more clearly the symptoms on these different hosts that might be definitely attributed to leaf hoppers and to observe further the effect of these injuries on different varieties or strains of the same host plant.

The experimental work at the Arlington Experiment Farm was conducted chiefly in cheesecloth cages 2 feet square by 3 feet in height. These provided some shade, but since the inclosed plants which were kept free from leaf hoppers apparently were only slightly affected by the reduced lighting, this factor is considered of little importance in interpreting results. In many of the plantings on the Arlington farm, especially in a large plot of Virginia black cowpeas, it was found that there was a heavy infestation of *Empoasca fabae* in

areas where other insects as well as plant diseases were extremely rare. This gave further opportunity to compare the symptoms produced on plants in the slight shade of the cheesecloth cages with those on plants under open field conditions, without the usual complications of other insect injuries or symptoms of diseases. For some of the work a smaller type of cage was used, consisting of a cheesecloth

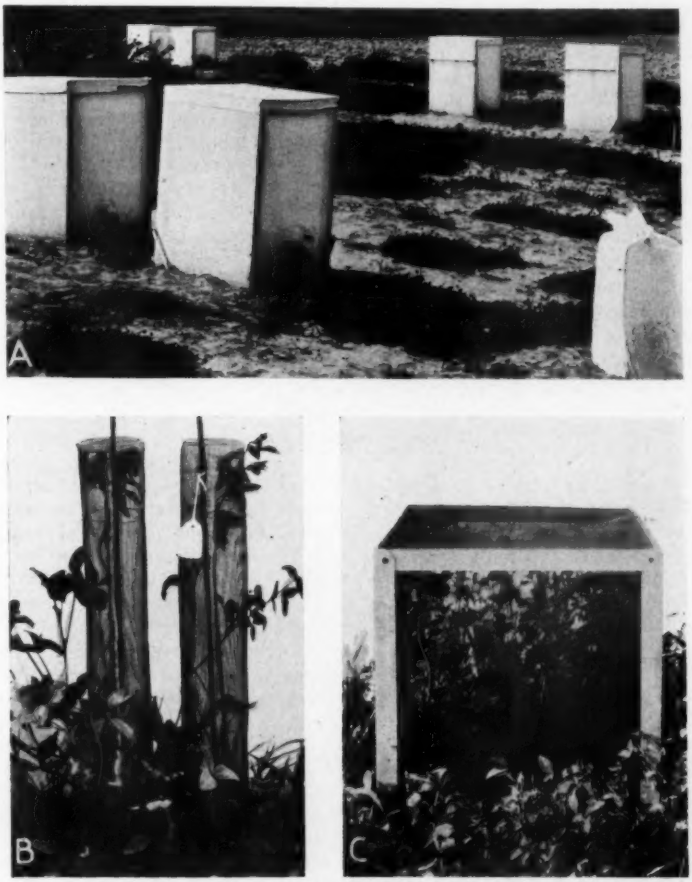


FIGURE 1.—Types of cages used in experimental work: A, At Arlington Experiment Farm, Rosslyn, Va.; B and C, at Illinois Agricultural Experiment Station, Urbana, Ill.

collar supported by wire, with the lower band of cloth buried in the soil and the top tied together in a bunch. This type had some advantages, but there was more shading, due to overlapping and bunching of the cloth at the top. These two types of cages are shown in Figure 1, A.

For most of the work, old plants in the legume nursery of the Office of Forage Crops were used. These plants had already shown

symptoms of leaf-hopper injury, but were cut back near the ground and dusted with nicotine dust before being covered with the cages. Therefore, these hopper-free plants were not checks in the sense of having been always kept free from hoppers. In other tests, such as those with cowpeas, beans, and red clover, seeds were planted within the cages so that the plants were always protected. All check cages were examined carefully at frequent intervals, to guard against any accidental infestation arising from eggs deposited on the plants near the ground previous to the time they were cut back, or against any other possible source of contamination.

Although there are several species of leaf hoppers that commonly feed on legumes, the present study was confined to the potato leaf hopper and the injuries that it produces. Hereafter, unless otherwise stated, where the terms "leaf hopper" and "hopper" are used, reference is made specifically to *Empoasca fabae*.[4] Both adults and nymphs used in the cages were collected in the field from legumes already showing symptoms of injury. No attempt was made to raise hoppers that might feed on legumes without producing disease symptoms.

The methods used in the experimental work at Urbana, Ill., were substantially the same as those followed at the Arlington Experiment Farm. Cages were covered with 24-mesh wire screening instead of the cheesecloth covering used at Arlington. (Fig. 1, B and C.) In the greenhouse work potted plants were employed, but for all other tests plants growing in regular field plantings were utilized.

INJURY PRODUCED

Year after year there has been observed a characteristic discoloration and dwarfing of many leguminous forage crops during the hot dry periods of summer. Perhaps the association of these symptoms with heat and drought has been in a measure responsible for the tendency to attribute all to "the weather." At any rate this unthrifty condition has generally received little attention from plant scientists, although it has obviously been correlated with lamentably decreased yields from many of these plants. In alfalfa it has for many years been referred to as yellows or yellow top, but a similar condition on clovers or other legumes has not been specifically designated. It is probable that it has usually been largely confused with drought injury, yellowing due to absence of nodule-forming bacteria, mosaic, so-called sun scald, or physiological response to hot weather.

Although varying in degree with different species or with different varieties within a species, both as to symptoms and as to total damage, the injury produced by an attack of leaf hoppers is of much the same type on all the various hosts. In all susceptible plants under observation there was a noticeable dwarfing of leaves, petioles, and stems as well as a decided reduction in floral development. Infested leaves frequently curl downward much as they are apt to do when injured by aphids. As a general rule, feeding by these insects results in some discoloration of the leaves. The discoloration in most cases con-

[4] Sample specimens were taken from the cages at the Arlington Experiment Farm and were identified as *Empoasca fabae* by A. A. Granovsky, of the University of Wisconsin. At Urbana, Ill., the leaf hoppers used in the cage experiments were mainly collected by an assistant of W. P. Flint, of the Illinois Natural History Survey. During the season 20 leaf hoppers of the same species as those used in cage infestations were identified by D. M. DeLong, of the Ohio State University, as *Empoasca fabae*.

forms closely to the leaf venation, extending downward along the veins and midrib or down the petiole. On the blade of the leaf a roughly triangular area is affected, extending from the leaf margin inward to a point on a vein or the midrib.

In most cases the injured area extends inward much farther on the proximal than on the distal side of the lateral vein, as shown in Figure 2. The change of color may be nothing more than a slight yellowing, but it may develop into a pronounced yellow, red, purple, or, in severe cases, the brown or "burn" of the tips or margins of the leaves, so commonly found on potato plants. Tissue showing the yellow or red symptoms may remain alive for a long time, and even badly dwarfed and discolored leaves may continue turgid and remain on the plant throughout the normal season. Browning is

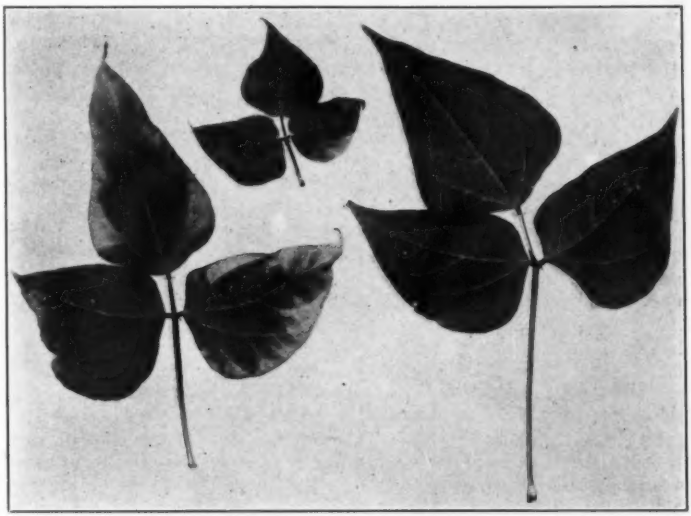


FIGURE 2.—Injury to garden beans caused by potato leaf hoppers. The two leaves at left from plant grown in hopper-infested cage, leaf at right from plant grown in hopper-free cage. Note discoloration along margins of larger leaf at left. Crinkling, dwarfing, and shorter petioles are also evident in the two leaves from infested plants

frequently accompanied by an upward curling of the leaves similar to that associated with hopper burn of potatoes. Although this latter symptom is generally recognized as the most important damage caused by leaf hoppers, and indeed is frequently regarded as the only symptom of leaf-hopper injury, it is evident that on legumes, and probably on other hosts, there may be injuries of great importance without any extensive evidence of hopper burn.

In severe cases leaf-hopper injury may destroy the host. This is especially liable to occur when the plant is in the seedling stage or when it is recovering from mowing. It appears likely that many seedling stands of clover and alfalfa are severely thinned or even practically destroyed by attacks of leaf hoppers. Their damage caused by these insects may also greatly reduce stands of clover or alfalfa soon after hay is havested. In crops such as cowpeas or soy beans there appar-

ently is little loss in the field from killing of individual plants. By far the greatest loss caused by leaf hoppers among the forage crops is that due to the extreme dwarfing of the vegetative parts of the plant and the resulting reduction in hay yield, pasturage, or green manure. Correlated with this is the check to floral development and the consequent reduction in yield of seed. Preliminary observations indicate that, taken collectively, leaf-hopper injury in a large area of this country may be of far greater economic importance than that of any other single known pest of forage crops.

RESULTS OF EXPERIMENTS WITH VARIOUS HOST PLANTS

CLOVERS

During the summer of 1927 insect-proof cages were placed over plantings of red clover (*Trifolium pratense* L.), zigzag clover (*T. medium* L.), white or Dutch clover (*T. repens* L.), Ladino clover (*T. repens latum*), and alsike clover (*T. hybridum* L.). Seedlings and second-year red-clover plants were caged, but in the work with other species only old plants that had previously shown hopper injuries were used. In addition to the above species, *T. pannonicum* Jacq., *T. alexandrinum* L., and *T. fragiferum* L., were also observed in open field plantings for symptoms of leaf-hopper injury.

The symptoms of leaf-hopper injury are in general similar in the several clovers studied. (Pl. 1.) On some species of Trifolium the red coloration that follows leaf-hopper attacks is apparently more pronounced than in any of the other legumes. This reddening develops gradually and usually does not become pronounced for at least a week after the insect feeds, although the time required seemingly varies somewhat with climatic factors and with the condition of the host. In Ladino, alsike, and zigzag clovers this reddening is most conspicuous. It is most common near the tips or margins of the leaves, along the veins, on the stalks of the leaflets, and in the upper portion of the petiole. Frequently, especially on zigzag clover, it extends along the entire border of the leaf blade. It sometimes occurs as definite angular blotches or more or less as streaks conforming primarily to the venation.

Coincident with the reddening symptoms, there is usually a yellowing of the leaf. Just back of the reddened tip there is often a streak of yellow which gradually merges into the dark green of the basal portion of the leaf. Frequently parts of a leaf, or even an entire leaf, may turn yellow without any indication of the red pigment. This is especially likely to occur where young leaves are affected. It is

EXPLANATORY LEGEND FOR PLATE 1

Potato leaf-hopper injury on white-clover and red-clover leaves

A. Healthy Ladino-clover leaf of average size taken from caged plant free from leaf hoppers.

B–G. Ladino-clover leaves taken from plants in adjacent cage heavily infested with potato leaf hoppers. Note dwarfing as compared with leaf A from check cage.

B. Slight marginal browning of the leaflets of an opening leaf and reddening of the upper portion of the petiole.

C. Withered and browned leaf with leaflets folded together but petiole still green.

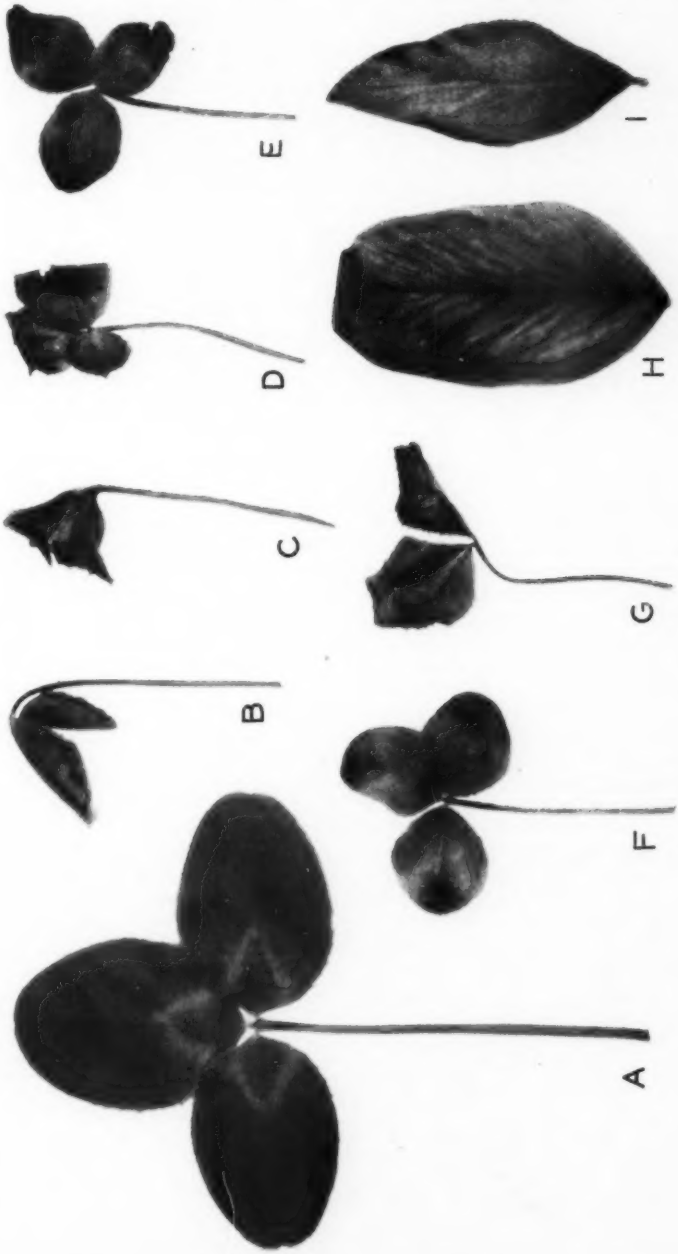
D. Typical tipburn with curling of the tips of each leaflet.

E. Reddening of the tips of leaflets with extreme tips turning brown. Upper part of petiole has turned red, but note green tissue both above and below this section.

F. Typical red and yellow banding as found on leaves of Ladino and white clover. Note red at tip, then yellow, and below the normal leaf green. Also note red portion of petiole.

G. Extreme discoloration and distortion of leaf and petiole. Note that this leaf has not wilted.

H, I. Leaflets of foreign red clover showing the typical reddening and yellowing produced by leaf hoppers on this host. Note the manner in which the coloring follows in general the venation, the tipburn, and curling in H, and the marginal browning in triangular areas on the right side and the irregular band of brown on the left side of I



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often the most common symptom on some of the strains of white clover and among the imported red clovers.

Although the red and yellow symptoms may occur alone, they are commonly accompanied by a browning or "burning" of the tips or margins of the leaves. This browning is similar to the well-known hopper burn of potatoes and therefore requires no elaborate description. In the infested cages and in the field entire young leaves were found to droop suddenly, turning to a grayish or brownish tint as they rapidly dried. Young succulent leaves are apparently unusually likely to collapse in this manner, particularly during periods of high temperature and drying winds. One may often find leaves of clover showing a tip burn, with a band of red below the browned portion, a band of yellow below the red, and the base of the leaf still holding the healthy green color. In a large planting of clover, especially Ladino, heavily infested with leaf hoppers there is often a beautiful display of brilliant autumnal coloring, which, as most of the leaves are killed, may rapidly change to a dull brown, as though injured by fire or extreme drought.

Experiments at Urbana, Ill.[5]

Experiments similar to those conducted in 1926 (*13*) were repeated in 1927 at Urbana, Ill., potted plants of Tennessee anthracnose-resistant and Italian red clover being used. On June 17 five mature potato leaf hoppers were placed in a cage containing two seedling plants with five or six leaves per plant. Four days after infestation one leaf on the Tennessee and three on the Italian plant had been killed. The other leaves on the Italian plant showed typical symptoms of leaf-hopper injury. Within two weeks this plant was dead, but three out of seven leaves of the Tennessee clover still were alive. Two weeks later the Tennessee plant was dead. The check plants in the hopper-free cage remained healthy throughout the experiment.

A test similar to the above was conducted simultaneously with older plants, 13 inches in height, which were producing flower stems. A cage containing two plants of the Tennessee strain and one Italian plant was infested with 20 leaf hoppers. Within two weeks, more than half the leaves on the Italian plant had been killed, and a week later it was dead. The two Tennessee plants showed a loss of only one-eighth of their leaves in two weeks and one-fourth in three weeks. Within four weeks (fig. 3) one of these plants was dead and 90 per cent of the leaves of the other plant had been killed. This third plant died the following week. The check plants remained healthy and came into full bloom during the course of the experiment.

At the conclusion of the experiment with seedling clover, the test was repeated with plants from the same source. The results were in general similar to those of the previous experiment. There was, however, more reddening of the leaves in this series, together with a more gradual browning and killing.

Duplicate cages (fig. 1, C) were placed in plots of red clover in field plantings to determine what effect an infestation of leaf hoppers would have on plants under field conditions. For this series, plots planted in August, 1926, with French, Rumanian, and Ohio seed

[5] Conducted by the junior writer in cooperation with W. P. Flint, the Illinois Natural History Survey, and the Illinois Agricultural Experiment Station.

were used. Hay had been removed in June, and when the experiment began the new growth was about 3 inches high. In addition to these three, a planting of Italian red-clover seed, made on an adjacent field March 1, 1927, was included in the experiment. In this fourth plot the plants were about 5 inches high. Leaf hoppers were removed from the plants that were covered with the cages, and on July 15 one cage on each plot was infested with adult leaf hoppers. The other cage was kept free from hoppers.

The symptoms noted in the field cages were similar to those observed in the caged pot cultures. Discoloration and killing of the leaves and dwarfing of the plants soon followed heavy infestation with leaf hoppers. Damage was most evident in the Italian plot, but the plants in the French and Rumanian plots were also severely injured. The plants in the Ohio plot, however, showed only a slight reddening during the entire period of the test. The flower-head development was reduced by the leaf hoppers, but the number of flower stems starting from the base of the plant was increased. The plants in the hopper-free cage on each plot grew rapidly and remained

FIGURE 3.—Injury to old plants of red clover, caused by potato leaf hoppers: A, B, E, and F are Tennessee anthracnose-resistant red clover; C and D, Italian red clover. Photographed 26 days after leaf hoppers were first placed in the cage

free from the reddening, yellowing, or burning so noticeable in the other cages.

One healthy red-clover plant in the field planting from French seed was selected for a test to determine what effect a heavy infestation of one part might have on the rest of the plant. Two wire-screen cylinders 20 inches long and 2 inches in diameter, were placed in an upright position above the plant. Into each of these cylinders was placed a single stem. (Fig. 1, B.) One cylinder was infested August 3 with one mature hopper and two nymphs, whereas the other was kept free from insects. In 10 days one leaflet on the stem in the infested cylinder was red. The injury increased until within six weeks all the leaves were killed. The leaves on the stem in the hopper-free cylinder remained green and healthy. During the course of this experiment the hopper-infested stem increased in length only 3 inches, whereas the hopper-free stem increased in length 24 inches.

In the field plantings where red-clover seed of different origin was being tested, it was apparent that after the first cutting there was a greater loss of plants among the imported stock than in the plots sown with home-grown seed. The plants in 3 linear rods of drilled

rows in each plot were counted two weeks after the first crop was harvested. The percentages of dead plants are given in Table 1.

TABLE 1.—*Reduction in stand of mature red-clover plants, resulting from leaf-hopper infestation previous to the first cutting at Urbana, Ill., in 1927*

Seed of foreign origin			Seed of domestic origin		
Source of seed	Dead plants	Number of plots	Source of seed	Dead plants	Number of plots
	Per cent			*Per cent*	
French	41.3	33	Ohio	12.1	1
German	53.4	2	Tennessee	15.6	1
Rumanian	52.7	2	Oregon	15.4	1
Chilean	56.5	2	Virginia	13.3	1
Silesian	33.7	1	Minnesota	13.3	1
Italian	46.7	1	Idaho	17.5	1
Polish	52.4	2	Michigan	[a] 5.7	1
Average	[b] 43.6		Average	13.3	

[a] Thin stand in this plot resulted in more vigorous plants.
[b] Average of the 43 plots.

The results as given in Table 1 show that there was a much greater loss of plants from the imported than from the home-grown seed. During the season leaf hoppers were more numerous and their damage more conspicuous in the plots from imported seed. No serious infestation with other insects or diseases was observed in these plots, so it seems reasonable to assume that the reduction in stand was in a large measure due to injuries induced by the leaf hoppers. The differences in response to hopper injury shown in red clover of different origin in the greenhouse experiments further support this supposition.

At Jacksonville, Ill., alsike clover grown in an insect-proof cage during spring and early summer remained free from leaf-hopper injury, while all the plants in the rest of the field were conspicuously reddened, dwarfed, and otherwise damaged by these insects.

EXPERIMENTS AT ARLINGTON EXPERIMENT FARM

In the latter part of July, 1927, at the Arlington Experiment Farm, duplicate cheesecloth cages were placed in three plots of red clover planted in May with seed of Tennessee anthracnose-resistant, Italian, and French red clover. One cage on each plot was infested with potato leaf hoppers. The plants in these cages showed the characteristic symptoms of leaf-hopper injury. Infestation, however, was light and practically all the plants survived. More injury was noted in the Italian and French clover than in the Tennessee strain.

In the legume nursery duplicate cages were placed over plants of Ladino clover and a selection of white clover that appeared much less injured by hoppers. These two sets of cages were on adjoining rows, so environmental conditions were approximately the same for each. Mature leaves were all removed and one cage of each pair was infested with about 40 hoppers and the other kept free from insects. In both infested cages the new growth soon showed the red and yellow symptoms of hopper injury. During the unfavorable periods in August the hoppers repeatedly disappeared from the white-clover cage, and it was therefore necessary to restock this cage with about 40 hoppers six times during the month The cage was carefully

examined many times in an effort to locate any predacious insect, but none was found that might account for this loss. The first infestation of the Ladino clover was sufficient for the season. By the middle of August there was a marked difference in the responses of the two clovers. The white clover showed unmistakable symptoms of injury, but by no means as pronounced as those on the Ladino clover. The red and yellow symptoms were very conspicuous in both infested cages. In the white-clover cage dwarfing and reduction in number of flower heads were apparent. In the Ladino-clover cage growth was soon stopped and the leaves rapidly withered and turned brown (Fig. 4.) No flowers were produced in this cage. At the end of the season no hoppers were found in the white-clover cage, but there were many adults and nymphs in the Ladino cage even though most of the leaves were dead and food was therefore scarce.

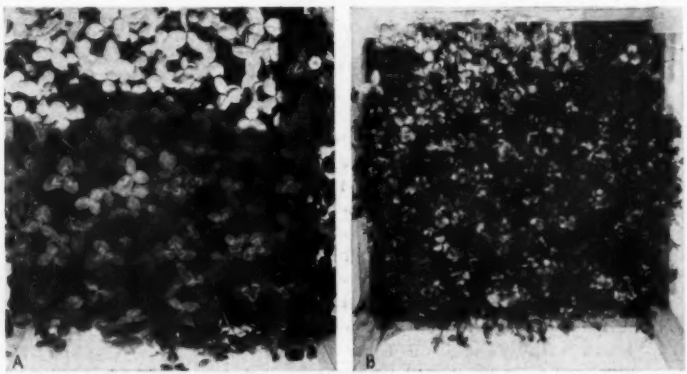

FIGURE 4.—Injury to Ladino clover caused by potato leaf hoppers: A, Check cage, inclosed in cheesecloth and kept free from leaf hoppers; B, Ladino clover in cage infested with potato leaf hoppers. Many leaves were killed and all others showed tipburn, reddening, or yellowing due to injuries by these insects. Note the dwarfing of the leaves

In another pair of cages in a Ladino-clover planting the check cage was accidentally placed over a colony of slugs. Since this gave an opportunity to compare mere feeding injury with leaf-hopper injury, the slugs were allowed to remain. In one cage every leaf was partially eaten and a great many were entirely consumed. In spite of this continued feeding and great reduction of the photosynthetic area, these plants continued to produce new vigorous leaves which apparently developed normally, until they in turn were eaten by the slugs. The plants in the hopper-infested cage, however, soon showed decided hopper injuries. Even with a larger total area of living leaf tissue than was left in the other cage, these plants gradually weakened and many died. Weeds soon replaced the clover in this cage, but in the other cage the heavy covering of new growth of clover seemed to keep most of the weeds in check. The slugs apparently preferred the Ladino clover to the weeds in the cage, so this difference of weed growth could not be attributed to a check of weed plants by the heavy infestation of slugs. Only a small number of plants survived the season in the hopper-infested cage, but practically all were alive when the cage was removed from the other group of plants in September.

Duplicate cages were placed over plants in a nursery row of zigzag clover. In the hopper-infested cage the red coloring was soon very conspicuous. There was also much yellowing and dwarfing due to leaf-hopper attack. Hoppers appeared to thrive on this clover, but they did not reproduce as freely as they had on the Ladino clover. The clover in the check cage was entirely free from any red or yellow symptoms.

Small cages were placed over new seedings of Italian and Tennessee red clover the third week in August. When the seedlings emerged, one cage over each of the two plantings was infested with potato leaf hoppers and the others were left as checks. In two weeks much injury was apparent on both the Italian and Tennessee clover, but there was slightly less on the latter. Results were partly confused by a severe attack of powdery mildew, which was much worse on the Tennessee clover.

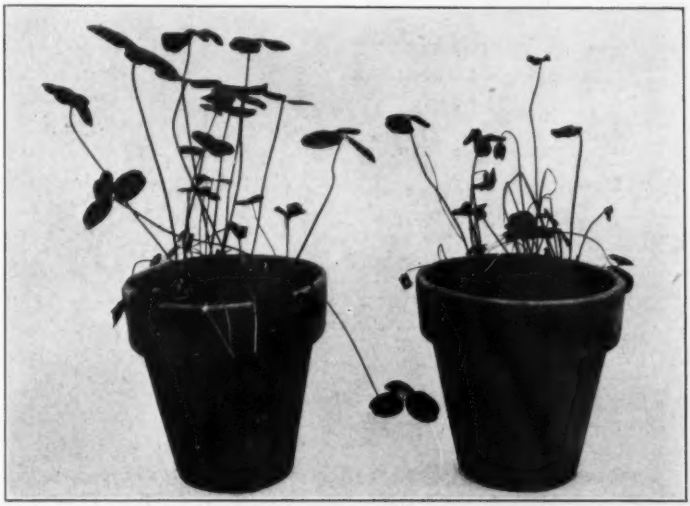

FIGURE 5.—Injury to English red-clover seedlings caused by potato leaf hoppers. Check plants at the left. The pot at the right contained five plants, on each of which was placed one potato leaf hopper in the second instar. The hoppers were allowed to feed on the plants during the remainder of their nymphal development (10 days). Many of the leaves were killed, and all showed injury

In the greenhouse at Madison, Wis.,[6] one potato leaf-hopper nymph in the second instar was placed on each of five English red-clover seedlings and kept there until it reached maturity. The temperature of the greenhouse was kept at 25° C. Within 24 hours wilting was evident on some leaves, and during the 10 days that the hoppers fed on them all the leaves were injured, and most of them were killed. (Fig. 5.)

ALFALFA

The symptoms of leaf-hopper injury on alfalfa (*Medicago sativa* L.), known as yellow top, yellows, or yellowing, have been observed for many years in this country (*17*), but apparently have never been

[6] In cooperation with A. A. Granovsky.

reported elsewhere. As compared with the symptoms on clover, there is a much more general development of the yellow and less of the red pigment. There is, however, a noticeable reddening, bronzing, or purpling of badly injured leaves, especially on the under part and along the midrib and veins. (Fig. 6.) Tipburn is common, but by far the most conspicuous symptoms are the yellowing, bleaching, and dwarfing. There is usually an abnormal development of lateral branches, which, together with the reduced length of internodes, gives the plant a rosette appearance.

Alfalfa fields are frequently so badly stunted from yellow top that there is scarcely enough hay to justify harvesting the crop. Nevertheless these stands after cutting may apparently recover

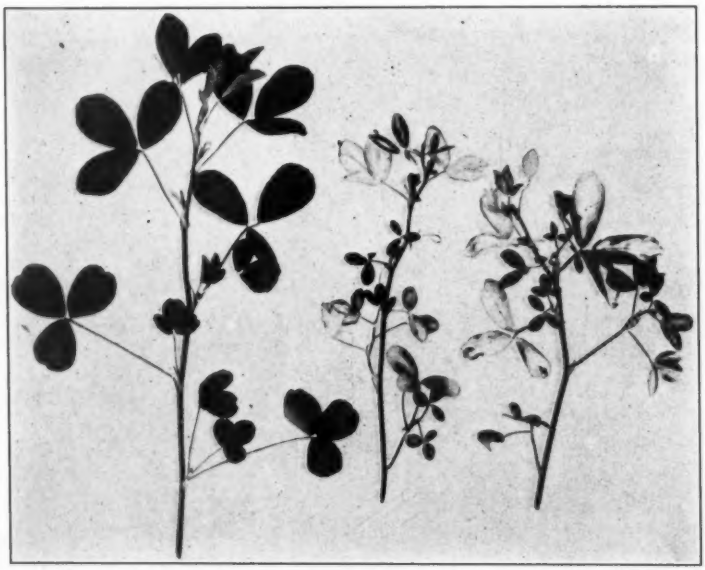

FIGURE 6.—Yellow top of alfalfa. Tips of branches taken from caged alfalfa plant shown in Figure 7. At left, upper portion of a branch grown in hopper-free cage. The two branches at right from hopper-infested cage. Note discoloration (yellowing or bronzing), dwarfing of new leaves and petioles, and also development of lateral shoots on the infested branches. Lower internodes of the two infested branches represent normal length, as these developed before insects were placed in the cage

entirely and produce a heavy crop of hay. Some experimental plantings of alfalfa at the Arlington farm have been kept under observation for the last three years. Each summer these plants have been badly yellowed and so stunted that most of the stems did not develop more than 6 or 8 inches in height. In spite of this, most of the plants recovered each fall after the diseased tops had been removed. A large number of the plants are still alive and appear to grow normally during the season when leaf hoppers are not present.

To determine whether the feeding of leaf hoppers on one part of a plant would have a deleterious effect on another part kept free from hoppers, three vigorous alfalfa plants that had been slightly checked

by yellow top were cut and the new shoots of each were covered with two cheesecloth cages. Half of the new stems of each plant were allowed to develop in a hopper-free cage and the other half were kept in a hopper-infested cage. Leaf hoppers were not introduced into the latter cages until the new shoots were 3 to 5 inches tall. Then several nymphs and mature hoppers were inserted, and these soon checked the growth of the shoots on which they were feeding. The other half of each plant continued to develop in an apparently normal manner. In four weeks the cages were removed and the internodes were measured. On the healthy side the mature internodes varied in length from 2 to 2½ inches. On the stems in the hopper-infested cage they averaged 2, 2½, 1½, 1, ½, ½ inches, respectively, from basal internode to tip. The first and second internodes were the same length in the check and in the infested stems, for hoppers were not introduced till that part of the stem was practically mature. On the healthy side just twice as many nodes had been produced as on the hopper-infested side. The reduction in the number of nodes and the checking of the normal elongation of the internodes produced a decided difference in length of branches in the two cages. (Fig. 7.) The healthy branches showed little or no tendency to develop lateral branches, whereas in the hopper-infested cages there was a marked development of lateral shoots. This increased number of branches is evident in the infested portions of the plant shown in Figures 6 and 7.

FIGURE 7.—Injury to alfalfa caused by potato leaf hoppers. A mature alfalfa plant was cut back within 2 inches of the soil and covered with two cheesecloth cages. Half of the plant (left) was kept free from leaf hoppers, while the other half (right) was infested with both nymphs and adults. The new growth had become 3 to 5 inches tall before hoppers were placed in the cage. Note the increased number of shoots and the dwarfing in the infested half and the absence of these in the uninfested half of this plant

SWEET CLOVER

Late in July, Anselm T. Stone, of the Office of Forage Crops, called attention to a planting of sweet clover (*Melilotus suaveolens* Ledeb.) that he had sown March 29, 1927, and in which growth seemed to have been suddenly checked during July. An examination of the more recent growth revealed the fact that the latest internodes and petioles were unduly short and that the leaves were much smaller than should normally have been the case. Throughout the field there was a yellow cast produced by the yellowing of the tips of large numbers of leaves. This same condition has been observed at Urbana and elsewhere. The unthrifty color, while quite noticeable, by no means approached the decided discoloration so characteristic of a field of alfalfa affected with yellow top. The same condition was found to a somewhat greater or less degree in the other plantings of sweet clover, both yellow and white (*M. officinalis* L. and *M. alba* Waldst. and Kit.).

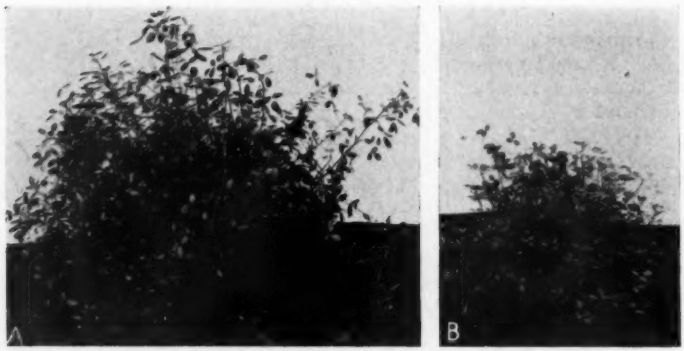

FIGURE 8.—Injury to sweet clover caused by potato leaf hoppers: A, Four plants grown in hopper-free cage; B, four plants grown in hopper-infested cage

At the Arlington Experiment Farm a number of plants of the yellow sweet clover (*Melilotus suaveolens*) were cut back to a height of 6 inches and covered with two cheesecloth cages. One of these cages was kept free from insects and the other infested with leaf hoppers. In two weeks, when the new shoots were well started, the difference between the plants in the two cages was quite evident. Those in the hopper-free cage recovered rapidly and produced a heavy growth of dark, healthy foliage. The new branches in the hopper-infested cage were decidedly stunted. Many of the leaves showed a slight yellowing of the tips, and in some there appeared the browning and curling so characteristic of hopper burn. Some of the branches wilted suddenly. Of the four plants in this cage there was one on which the leaf hoppers appeared to concentrate, and one after another the branches of this plant wilted, until within the course of four weeks the entire plant was killed. The other plants, however, continued to grow, and during the period when leaf hoppers did not thrive in the cage they were able to recover quickly. At the end of the summer there was a decided difference in total growth in the two cages (fig. 8), in spite of the fact that this experiment was

conducted during the weeks when the weather conditions were apparently unfavorable for leaf hoppers. The symptoms in the cage so closely resembled those observed in the open as to leave little doubt that the yellowing and dwarfing of Melilotus during July had been in a large measure due to injuries resulting from the fairly heavy infestation of leaf hoppers.

The cages were removed from the plants the third week in September, and thereafter they were left uncovered. Leaf hoppers after this time were extremely scarce in this field. This species of Melilotus is normally resistant to frost, but on November 15 Stone observed that frost had killed all the leaves on the three plants that had been inclosed in the hopper-infested cage. The leaves on the plants that had been inclosed during late summer in the hopper-free cage, only 2 feet away, showed no sign of frost injury at that time, nor until a month later.

COWPEAS AND SOY BEANS

In the extensive plantings of a large number of varieties of cowpeas and soy beans at the Arlington Experiment Farm there was found, during July, a decidedly stunted condition of the plants, together with a yellowing and curling of the leaves, and a widespread occurrence of the tipburn so suggestive of hopper injury. This unthrifty condition was much more striking on some varieties than on others, and wherever it was most conspicuous there the potato leaf hopper was found in greatest abundance.

Three varieties of cowpeas were sown under cheesecloth cages at Arlington and kept free from insects until the first trifoliolate leaf had matured. Leaf hoppers were then introduced into one cage, and the other cage remained as a check. For some time the weather seemed unfavorable for leaf hoppers and it was necessary to replace those that died in the cage. In a few days some of the younger leaves in the hopper-infested cage began to show the dwarfing and yellowing noticed in the field, and the older leaves showed the large angular blotches of yellow as well as the browning and curling at the tips. Although not so pronounced as those in the field, probably due chiefly to the smaller number of leaf hoppers on the caged plants, the symptoms shown in the hopper-infested cage were unmistakably those so widespread throughout the plantings at the Arlington farm. (Fig. 9.)

Cages were also placed over plants of Tarheel Black and Mammoth Yellow soy beans, and on the Mammoth Yellow variety in the infested cage the typical yellowing and curling symptoms were produced.

Affected plants have a yellow color very much like that appearing in cowpeas or soy beans growing in the absence of nodule-forming bacteria. A heavy infestation invariably results in a decided checking of the growth of the plant, with a pronounced decrease in length of petiole and size of leaf blade. In some instances the entire plant may have a yellow, unhealthy appearance, while in others the yellowing is limited to the tip of a leaf or to an area more or less triangular in shape. This angular yellowed area is usually bounded by the veins and extends nearer to the midrib on the proximal side of the lateral vein than on the distal side. Parts of a leaf, most commonly the tip or the area along the margins, may wilt, dry out, and turn brown. This type of injury has probably been frequently regarded

as sunburn, for leaves in the first wilting and drying stage as well as those in the later brown stage have much the appearance of having been scalded. As growing leaves are attacked, they curl downward and take the shape of an inverted cup. Along the veins and midribs of such leaves the epidermis is frequently badly torn. This condition is especially common along the fleshy midrib, and one often finds leaves on which practically all the lower part of the midrib is brown, due apparently to innumerable lacerations produced by the insects in the process of oviposition. In damp weather this injured tissue is frequently invaded by saprophytic fungi and may appear as if diseased by them.

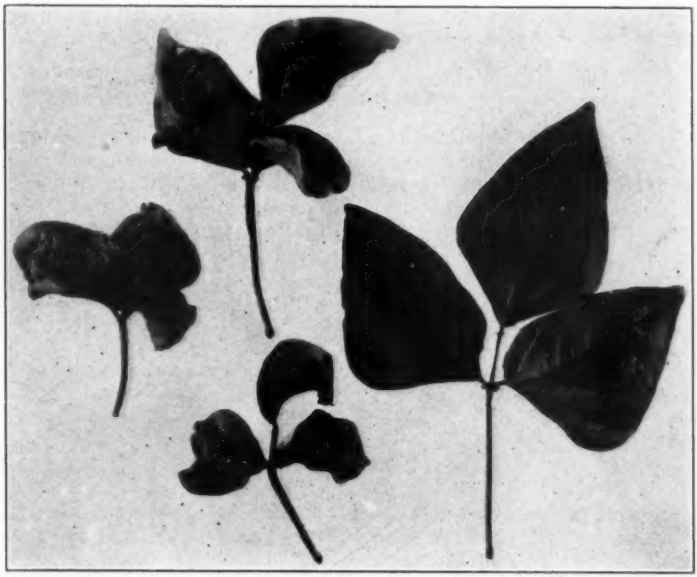

FIGURE 9.—Injury to cowpea caused by potato leaf hoppers. The three leaves at the left show curling and dwarfing due to heavy infestation. The leaf at the right was taken from the same plant as those at the left, but it was grown after the insects had disappeared from the plant. The injured leaflets remained on the plant and all four leaves were removed and photographed the same day. The plant was not caged

LOTUS

Some nursery rows of *Lotus corniculatus* L. for several years have been observed to turn an unhealthy yellowish or brown color in midsummer. During the summer of 1926 these rows were examined for insects, and it was found that they were heavily infested with the potato leaf hopper. The naturally small leaves were much smaller than those found on spring and fall growth. These tiny blades showed yellowing and bronzing symptoms similar to those observed as hopper injury on alfalfa. There was also a heavy loss of photosynthetic surface due to tipburn and to browning of entire leaves or stems. During the summer of 1927 the same condition was again observed in all the rows of lotus.

A number of plants in one row at the Arlington Experiment Farm were cut back within an inch of the soil level and on July 27 these were inclosed in two cheesecloth cages. As soon as new growth had well started, leaf hoppers were introduced into one of the cages. The other was kept free from insects. Within two days after hoppers were placed in the cage some of the leaves and tips of the new shoots began to wilt. These wilted parts usually soon dried and became

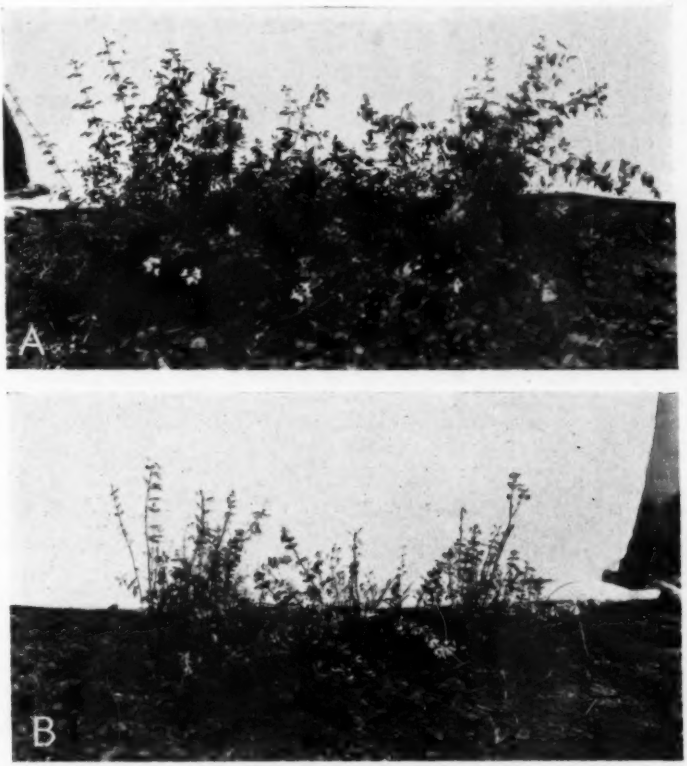

FIGURE 10.—Injury to *Lotus corniculatus* caused by potato leaf hoppers: A, Plants grown in hopper-free cage; B, plants grown in hopper-infested cage

brown. Growth of the other branches was quickly checked, and within a week the leaves began to show the yellowing symptom so common in the plants out in the open. This yellowing was most pronounced on the tips, as is the case in other plants, and with it occurred the characteristic tipburn. Later there developed a decided bronzing or reddening of the leaves, petioles, and even stems. Flowers failed to develop in the hopper-infested cage, where as several clusters of blossoms were produced in the hopper-free cage beside it. (Fig. 10.)

BEANS

During July, when leaf-hopper injury was so much in evidence at the Arlington farm on many of the forage legumes, L. L. Harter called attention to a similar type of injury on many of his plantings of beans (*Phaseolus vulgaris* L.). Throughout the affected area the potato leaf hopper was found to be generally distributed, and among the different varieties there was a direct relationship between the number of these insects on the plants and the severity of the damage.

Seed of the Bountiful and Tennessee Green Pod varieties were planted in the forage-legume nursery at Arlington. These were covered immediately with cheesecloth cages and kept free from insects until the plants were well developed. One cage was then infested with leaf hoppers and the other kept free from insects. In this case, as with cowpeas, the unfavorable weather conditions so interfered with the work that the plants were well grown before leaf hoppers could be gathered and kept alive in the cage in numbers sufficient to check all the plants. However, here also symptoms that were practically identical with those found in the open under field conditions were produced in the hopper-infested cage.

Tip and marginal burnings of the leaves resulting from attacks by leaf hoppers are symptoms that have frequently attracted the attention of other workers (*3*). The yellowing, curling, browning of midrib and veins, dwarfing, and other symptoms as described for cowpeas occur also on beans. One of the interesting symptoms observed in the caged plants, as well as in the field, is the dwarfing and curling of the young leaves and the crinkling of the surface. Such leaves at times show little or no loss of dark-green color.

MISCELLANEOUS LEGUMES

Typical symptoms of leaf-hopper injury have been observed on other legumes. Tipburn and slight yellowing have been seen on leaves of several species of Lespedeza. Usually leaf hoppers are not abundant on these hosts, and injury is not often conspicuous. Some species are more noticeably affected than others. Kidney vetch (*Anthyllis vulneraria* L.) showed only slight yellowing and browning in a planting observed in the summer of 1927. Peanut (*Arachis hypogaea* L.) in the greenhouse proved to be a favorable host and showed typical tipburn and some yellowing. Sainfoin (*Onobrychis viciaefolia* Scop.) and species of Vicia have also shown similar symptoms.

VARIETAL DIFFERENCES IN RESISTANCE

Some varieties of apples and potatoes have been repeatedly observed to show greater damage from leaf hoppers than other varieties growing in adjoining rows (*1*, *8*). Thus Ball pointed out that "invariably the Green Mountain plants, even where occurring in the same hill with the Rurals, were showing considerable injury, while the Rurals even with the leaves interlacing were comparatively free." Later observations, however, apparently led him to question this difference, owing to the fact "that most of this variation was due to a difference in the time that these plants developed sufficient foliage to furnish a place for egg deposition for the spring brood of adults."

The behavior of clovers and other legumes, both in cages and in the field, gives every indication of varietal differences regardless of the

quantity of foliage available for deposition of eggs. As was noted in the case of potatoes, there are times when the apparently more resistant varieties of legumes are severely damaged by hoppers. In a number of legumes planted at the same time and with approximately the same foliar development, the insects were found to be more numerous and the damage caused by them much more severe on certain varieties than on others grown in adjoining rows. The number of hoppers

FIGURE 11.—Injury to Ladino clover caused by potato leaf hoppers: A, Plants grown in hopper-infested cage; B, plants grown in hopper-free cage. Compare severity of damage as evident in upper plot with a similar hopper-infested cage of white clover, Figure 12

inserted in the cage with the "resistant" variety of white clover was many times that used in the cage with the "susceptible" Ladino clover. However, at the end of the season there was still a heavy infestation of hoppers in this latter cage, even though the damage to the plants had reduced their food supply almost to starvation proportions. (Figs. 11 and 12.) In the cage with the resistant variety the hoppers failed to propagate and when the cage was finally removed at the end of the season none could be found on the plants.

In clovers there is a decided difference in the severity of leaf-hopper injury both among the various species and among varieties or strains within a species. The common alsike, like Ladino clover, is usually badly injured. White clover ordinarily is less seriously damaged, and American-grown red clover is least damaged of the common agricultural clovers. Zigzag clover is usually badly damaged. Crimson clover suffers little, owing apparently to the fact that it is ordinarily planted at a time when heavy leaf-hopper infestations can be largely

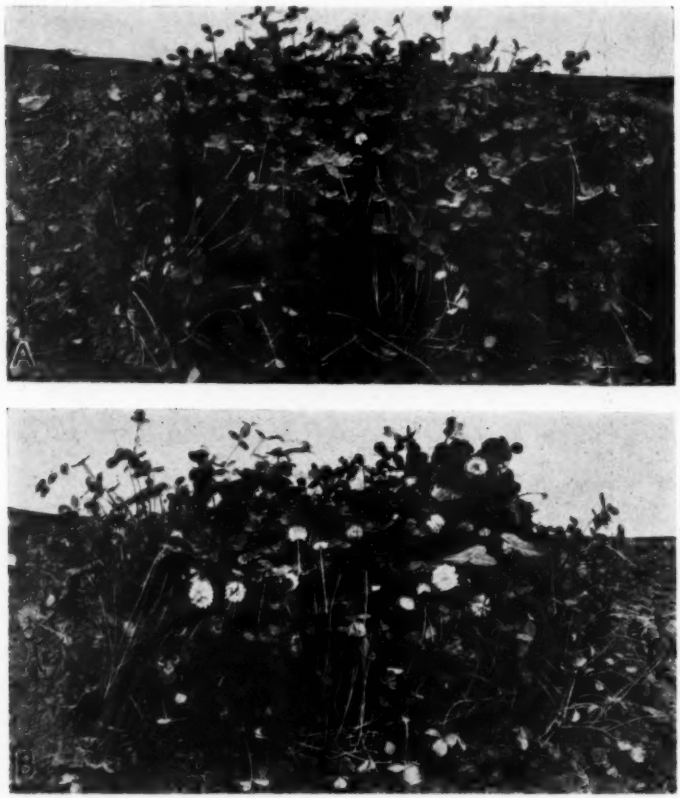

FIGURE 12.—Injury to white clover caused by potato leaf hoppers: A, Plants grown in hopper-infested cage; B, plants grown in hopper-free cage. Compare with Figure 11. Note reduction in floral development in plot A

avoided, and is harvested or plowed under before the hot period of summer when leaf hoppers are abundant. Hungarian clover (*Trifolium pannonicum*) is only slightly damaged, berseem (*T. alexandrinum*) becomes yellowed and bronzed much like alfalfa, and strawberry clover (*T. fragiferum*) is reddened and tipburned.

Red clover responds differently to leaf-hopper attacks, depending on whether the seed has been produced in North America or whether it has been recently imported. For several years clover plantings of

European or Chilean seed have become yellow and generally unhealthy in appearance during midsummer throughout most of the clover belt. This difference has generally been explained as being due to the fact that the imported clover had not become acclimated and could not withstand the extreme conditions of our summer season. Of the imported seed, the Italian stock usually is most conspicuously affected. Frequently there is an attack of anthracnose (*Colletotrichum trifolii* Bain and Essary) during the summer, and since Italian stock is most susceptible to this disease, it is difficult to determine in the field just how much of the reduction in growth is due solely to leaf-hopper injury.

During the summer of 1927 the plantings of red clover at both the Virginia Agricultural Experiment Station, Blacksburg, Va., and the Illinois Agricultural Experiment Station, Urbana, Ill., presented a striking example of the difference in response to hopper injury of plants grown from native and those grown from foreign seed. All the plots of European and Chilean seed were badly yellowed and stunted, whereas all the plots of native-grown seed remained a healthy green. The plants from imported seed produced relatively few flower heads, and few of these were of normal size, whereas the domestic plants all produced an abundance of large well-formed flower heads. This difference was apparently due chiefly to the attacks of leaf hoppers, for anthracnose and other diseases were much less prevalent than usual. This same difference in color and vigor of growth as well as in flower-head development has been observed repeatedly at the Arlington farm and on many experimental plantings throughout the East and Middle West where red-clover nationality trials have been conducted. This varietal response has also been found to occur in plants inclosed in cheesecloth cages and infested with leaf hoppers. An American-grown red clover, Altaswede,[7] has been badly damaged by these insects. It has also been observed that mammoth clover ordinarily shows even less leaf-hopper injury than does the native medium red. Whether leaf hoppers are responsible for all or most of the failures of imported red clover has not yet been determined, but the evidence now available certainly indicates that they are an important contributing factor if not the chief cause of the losses in many States.

As has been repeatedly observed in other crops, a field of a "susceptible" clover or other legume may frequently be found that shows no evidence of injury, whereas other fields near by may be severely damaged.

The reason for this varietal difference in resistance is an interesting question which so far has not been answered. In red clover, as has been already pointed out (*13*), there is a direct correlation between hairiness of the leaves, petioles, and stems and resistance to hopper injury. All the European and South American red clovers imported into this country are smooth or appressed pubescent, whereas the red clover produced in this country and Canada is rough pubescent. (Fig. 13.) Hairy Peruvian alfalfa has been observed to be more resistant to yellow top than are the smooth varieties. Likewise the most susceptible varieties of cowpeas and soy beans apparently are those with the smoothest surface of stem and leaves.

[7] Altaswede is a selection from the Swedish Late and is of the smooth foreign type, not of the type commonly called American clover.

At the Arlington Farm a large planting of cowpeas of different varieties with alternate rows of Pekin soy beans presented an interesting contrast of severe leaf-hopper damage on the smooth varieties of cowpeas and practically no damage on the hairy soy-bean plants. Also in alternate-row plantings of Mammoth Yellow and Tarheel Black soy beans a striking difference was observed in the severe dwarfing and decided yellowing of the Mammoth Yellow and the almost total absence of injury on the Tarheel Black.

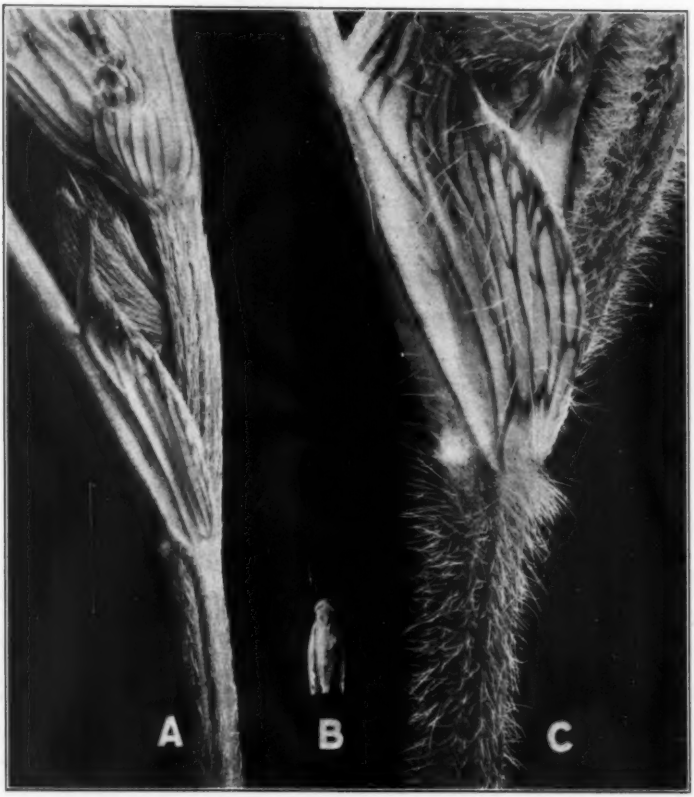

FIGURE 13.—Stems from relatively glabrous and pubescent red-clover plants and a mature leaf hopper (× 4): A, Stem from Italian plant; B, mature leaf hopper; C, stem from Tennessee plant

When hoppers from smooth leaves are placed on the hairy surface of such plants as American red clover they do not appear to be altogether at ease. In time, however, they seem to become accustomed to the hairy obstructions and may feed there and live for some time. When obliged to feed on such plants they are capable of causing the same type of injury that they produce on the smooth varieties. As has been shown, the smooth forms of red clover are more seriously injured by the leaf hopper than the hairy forms, and

the apparent preference in this case, as in others that have been recorded, of the adult females for smooth varieties, strongly suggests that differences in hairiness or other leaf structure may have some influence on oviposition. It was also noted that although both nymphs and mature hoppers are able to feed on hairy plants, there is a remarkable difference in the relative numbers of nymphs produced on different varieties. Possibly many of the newly hatched nymphs were trapped and killed among the leaf hairs before they were easily observed, as was found to be the case on potatoes (*5*). It was noted on legumes that the insects apparently do not avoid the tips of branches or opening leaves as they seem to do on potatoes. The tips of growing potato shoots, unlike such plants as Ladino clover are unusually hairy, which may account for this difference in response.

Although dense pubescence evidently serves as a mechanical obstruction which in some way interferes with the activities of the insects, there appears to be some factor involved other than or in addition to that of hairiness. Plants with succulent and fleshy veins or petioles, as cowpeas, seem to be preferred by the hoppers. The varieties of white clover that were studied under cages are both classed as smooth. Nevertheless, when these varieties were grown in the field with the smooth and hairy red clovers the damage to the white clover referred to as "resistant" was more nearly like that caused to the smooth red clover than to the hairy.

In some cases there was observed a difference in response which suggested actual resistance within the plant. The most striking case of this was in a greenhouse kept at 25° to 30° C. at Madison, Wis. Triumph potatoes were severely injured, whereas peanuts were only slightly injured by equally heavy infestations of hoppers. The insects apparently thrived on both plants, but the temperature and low humidity of the greenhouse were, of course, more favorable to peanuts than to the potatoes.

EFFECT OF WEATHER CONDITIONS ON LEAF-HOPPER INJURY

Leaf-hopper injury to legumes in most parts of this country occurs chiefly during the summer months. In the far South, however, it is reported as beginning in early spring and continuing into the fall.

The degree of injury to legumes varies closely with the fluctuations in the number of hoppers, although extreme heat and drought may greatly exaggerate the damage caused by a fairly light infestation. Throughout the eastern and mid-western clover belt, the first conspicuous symptoms on clover or alfalfa usually appear during the latter half of June, but in some years they may be fully a month earlier. Since the yellowing or reddening develops gradually, these particular symptoms may appear most noticeable in the field a short time after the hoppers have been most numerous. The wilting and browning of parts of plants during periods of heat and drought occur often within a day after hoppers feed on the plant, and therefore these symptoms are most common during the hot, dry periods when leaf hoppers are most numerous. After a heavy infestation of leaf hoppers had caused serious damage to crops, a period of unfavorable weather in August, 1927, so noticeably reduced the number of hoppers at the Arlington Farm and at Urbana that damage was thereafter negligible. Even badly dwarfed and discolored plants

quickly sent out new foliage which in many cases appeared to be normal in every way. This recovery has been repeatedly observed when a crop of alfalfa affected with yellow top has been harvested just before a period of rainy weather. Much of this difference in growth no doubt has been due to the difference in moisture available to the plant, but not all can be explained solely on the basis of water supply. In the plantings of cowpeas at the Arlington Farm it was noted that in many sections where the soil was moist and the plants had made a rank growth or were crowded with a heavy growth of grass or weeds the hoppers were extremely scarce as compared with sections in the same field which were on drier ground or where the stand was thinner than usual. In these low areas with dense vegetation some moisture frequently remained on the lower foliage all day during cloudy weather.

On the drier areas of the field where plants were more scattered the foliage dried rapidly, and there leaf hoppers appeared to thrive exceedingly well; but even in these places they disappeared rapidly during certain periods of wet weather. Alfalfa yellows has been associated with higher and drier parts of fields (*16*).

No experiments were conducted to determine the effect of rain or humidity on the welfare of the leaf hoppers. The evidence available from field observations indicates that the difference in severity of leaf-hopper damage in various parts of a field is influenced less by the quantity of water available to the plant (which might explain the more rapid drying out of foliage on plants growing in drier soil) than it is by the effect of moisture on the insects.

Observations in both field and cage studies suggested that atmospheric humidity or dew might, through their influence on parasitic fungi, exert an important influence on the leaf-hopper population and the consequent injury to legumes.

The increase in leaf-hopper population during hot, dry weather and the decrease during periods of abundant moisture, together with the more serious and more noticeable injury to plants during periods of most rapid transpiration, help to explain the rather general confusion of leaf-hopper injury with the damage actually resulting from drought. The correlation of leaf-hopper damage with relatively high temperature and low humidity is important in the production of such crops as clover and alfalfa. Red clover planted with a nurse crop is suddenly exposed to a most exacting period when the nurse crop is removed. Frequently by the time this occurs the soil moisture has been greatly depleted and the seedling clover plants are growing slowly, their partial shade has been removed, and conditions are made extremely favorable for leaf hoppers. A similar condition exists after hay harvest of clover or alfalfa. At such times the exposed crown and new shoots are badly damaged or killed, and leaf hoppers flourish in the thin stand.

CAUSE OF INJURY

The direct cause of the injury produced by leaf hoppers has not been definitely determined. It seems evident from repeated observations on legumes and other crops that the damage is not due simply to mechanical injury produced by the insect in puncturing the tissue or to the extracting of food from the plant. Observations such as those made in the hopper-infested cage of Ladino clover as contrasted

with those made on the slug-infested clover indicate that removal of food or reduction of photosynthetic area would not suffice to cause the severe injury associated with these insects. A single leaf hopper even in the early nymphal stages is capable of wilting a whole new shoot of such plants as lotus and clover. The same shoot might easily support many aphids without serious damage. Ball pointed out the differences in symptoms produced by insects of the same genus, in which the feeding process must be very similar, as indicating that the injury produced by the potato leaf hopper is specific for that particular species. General observations on legumes herein reported support those previously made on potatoes and apples in indicating that there must be either some substance transmitted from plant to plant or some insect secretion injected into the plant tissue to account for the rapid collapse of tissue to give the burning symptom or to produce the gradual discoloration so noticeable in clovers. These might be accounted for by the supposition that the injury is due to some peculiar virus or to some toxin or enzyme produced by the insect.

The slowly developing reddening and yellowing symptoms, as in clovers, together with the curling, dwarfing, and yellowing of the new growth, at once suggest the somewhat similar type of development of mosaic or other virus diseases of plants. The association of leaf hoppers with other virus diseases of plants also makes one question whether this, too, may not be of a similar nature. So far, apparently, no one has attempted—at least, none has reported—growing this species of leaf hopper free from the infectious principle. Other workers (*4*, *5*) have artificially produced symptoms resembling leaf-hopper injury by injecting into healthy plants juice from crushed leaves that showed leaf-hopper injury. No one has successfully carried this test further than a single transmission from the original injured tissue. Transmission of the above nature might be accomplished in the case of either a virus or a toxin. Experiments showed that half an alfalfa plant or individual branches of clover might be badly damaged by hoppers while the rest developed in an apparently normal manner. Similar experiments have been performed with other hosts. In addition to these experiments, the frequent observation that badly damaged plants apparently may recover entirely, even under conditions when leaf-hopper injury is developing near by, all make it difficult to explain this particular injury as due to a virus, at least with the present conception of a virus as associated with plant diseases.

The theory that appears most plausible (*20*, *p. 24*) on the basis of observations to date is that the insect when feeding injects into the tissue some chemical or enzymic toxin in a manner somewhat analogous to that found in plant gall-producing insects or in mosquitoes feeding on human tissue (not of malaria or other disease transmission). Such an injection might well explain the rapid wilting of young succulent tissue and its total collapse and death. The wilting very closely resembles the type of injury associated with certain vascular Fusarium diseases of plants, which in these latter cases has been reproduced by enzymes produced by the fungus. Likewise leaves may turn yellow but remain turgid under conditions that cause healthy leaves to become flaccid, suggesting the phenomenon seen in cabbage or other plants with vascular infection by Fusarium. In

many cases the damage is entirely local; often a petiole of clover where a nymph has been observed feeding will collapse and the upper portion dry out, whereas the lower section remains green, much as is the case with an infection with anthracnose. At other times the injury appears to be more generally diffused and less severe at any one point. This type was most noticeable during the cooler, moister periods more favorable for plant growth. Whatever is injected apparently is intimately associated with plant metabolism, and the extent and nature of the injury varies with the transpiration rate and other plant physiological processes.

At times an excessive infestation of leaf hoppers may so weaken even mature plants that they are unable to recover even though kept under favorable conditions and free from insects. Whether this is due to some movement of the toxic substance (or virus) into the roots or whether it is the secondary effect due to exhaustion of the food reserve in the root system is a point that has not been determined. At least part of this type of damage may be done by the insects feeding close to the crown as the new buds develop. This evidently is what happens in young seedlings of clover which may be quickly destroyed by a small number of hoppers. In such cases the injection of a poison might easily kill a plant, even though the poison be localized.

MIGRATION

At the Arlington farm during the summer of 1927 the plots of second-year red clover were located just across a narrow roadway from the new spring seeding of clover. Just before harvesting the old clover it was found that there was a fairly heavy infestation of mature leaf hoppers throughout the planting. When the crop was cut there was of course left on these plots little green material upon which they could feed, and for a time even this was covered with the drying hay. There was a rapid increase in the number of hoppers on the seedling plots across the road and after a week or more these plots showed much damage. A part of this increased damage was undoubtedly due to the natural increase in the number of leaf hoppers on the plots, but it is probable that it was also partly due to the migration of the insects from the plots where their food supply had been suddenly removed. The seedling crop remained throughout the season more heavily infested than the plots of old clover across the road, which indicated that there was no migration back to those plots. It has been observed frequently that there is a noticeable difference in severity of yellow top in neighboring plots of alfalfa which have been cut at different times. This has led to the belief that yellow top is a physiological disturbance induced by cutting at the wrong time. An explanation of this difference has been attempted by attributing it to some difference in reserve carbohydrate storage in roots (*10*). It appears that there may be a yellowing of alfalfa due to such causes, but it is also extremely likely that many of these differences may be explained by the variation in leaf-hopper population due to migration from plot to plot.

It is possible that in one season or locality the first plot cut might be more heavily infested, whereas under other conditions the reverse might be the case. This would depend to some extent on the availability of young succulent shoots, the stage of development of the hoppers when the plants are cut, the climatic conditions affecting

moisture on the taller plants and the shorter growth of the newly cut plot, and perhaps many other factors. Apparently such an inconsistency has been observed in different localities. The cutting of such crops as alfalfa and clovers results in a problem somewhat different, from the standpoint of hopper infestation, from that found in such crops as potatoes and beans where the growth is continuous until the plants are harvested or killed. The sudden removal of quantities of food material, leaving only the limited food supply of the new shoots, must have a decided influence on the severity of this type of injury. Such plants at those times are often greatly weakened, even without the presence of insect pests. If weather conditions following hay harvest are unfavorable for plant growth but at the same time are favorable for leaf-hopper propagation, the second crop will be of little value. On the other hand, if most of the insects have reached the adult stage by the time the hay is harvested, they are likely to migrate to other crops and leave few hoppers to interfere with the new growth.

SUMMARY

Field observations in 1926 and 1927 indicated that several forage-crop legumes suffered severely from attacks of potato leaf hopper (*Empoasca fabae* Harris).

In the United States the injuries caused by the leaf hopper to forage legumes appear to be worst throughout the clover belt and southward.

Experiments were conducted at the Arlington Experiment Farm, Rosslyn, Va., and at the Illinois Agricultural Experiment Station, Urbana, Ill., to determine the symptoms and the extent of the injuries produced by them on several hosts.

Plants in field plots and in greenhouse pot cultures were inclosed in duplicate cheesecloth or wire-netting cages. One cage was kept free from insects and the other infested with potato leaf hoppers. In these cage experiments there were included the following: Medium red clover (*Trifolium pratense*), white clover (*T. repens*), Ladino clover (*T. repens latum*), alsike clover (*T. hybridum*), zigzag clover (*T. medium*), alfalfa (*Medicago sativa*), sweet clover (*Melilotus suaveolens*), cowpea (*Vigna sinensis*), soy bean (*Soja max*), lotus (*Lotus corniculatus*), and garden bean (*Phaseolus vulgaris*).

Symptoms on legumes include the common tip and marginal browning known as hopper burn, as well as a conspicuous reddening, bronzing, and yellowing of leaves, petioles, and stems. There is usually a pronounced dwarfing of the plant, curling of leaves, and check in floral development. Symptoms vary somewhat on different hosts, and the characteristic response of each plant is described.

Yields of hay or forage may be seriously reduced by leaf hoppers. It is evident from the studies that the leaf hoppers may be an important factor in reducing or destroying stands of clover, alfalfa, and perhaps other forage crops. Seed production may also be decidedly restricted by them.

The observations here reported further indicate that the damage caused to plants by this insect is the result of some disturbance of the metabolic processes of the plant due to injection of an enzyme or toxin, which apparently remains more or less localized.

Some parts of a plant may be affected without evidence of injury to other parts. After a heavy leaf-hopper infestation plants have sometimes been observed to recover completely, but in many cases

the injury has proved to be permanent. A decided difference was observed in the degree of injury among the various legumes and also among different varieties within a species. In many cases greater resistance appeared to be correlated with more pronounced pubescense. Apparently, the susceptibility of a plant is largely accounted for by the preference of the insect for that individual, but some indications were noted of actual resistance within the plant to the injuries induced by this insect.

Plants succumb to leaf-hopper injury most rapidly in hot, dry weather. The greatest damage was observed during periods of heat and drought. This was probably due somewhat to the greater susceptibility of the plants, but much more to the increased activity of the insects during such periods. During excessive rainfall, lower temperatures, and high humidity the hoppers rapidly decreased in numbers.

The insects migrate when the food supply is limited, and a difference of a few days in the date of planting or the time of cutting may noticeably influence the damage they cause to a crop.

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FRUIT-BUD DEVELOPMENT IN THE TUNG-OIL TREE [1]

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The Chinese wood-oil or tung-oil tree, *Aleurites fordi* Hemsl., was introduced into this country from China in 1905 by the Office of Foreign Seed and Plant Introduction of the United States Department of Agriculture. Of the genus Aleurites five species have been described, namely, *A. fordi*, *A. cordata*, *A. moluccana*, and *A. trisperma*.

The investigations reported herein deal only with the species *A. fordi*, which is known in its native country as tung-shu or tung-yu-shu, literally, tung-oil tree. Commercially this is by far the most important species of the group, since it furnishes approximately 90 per cent of the tung oil of commerce.

The tung-oil industry of this country, which is limited to the Southern States, particularly to Florida, is of recent origin. The tree has not been long enough in cultivation in America for experience to demonstrate what cultural practices are best suited to insure high productivity. The various practices may be considered as affecting the crop in three rather distinct ways; they influence the growth of the tree, the differentiation of blossom buds, and the development of nuts from the blossoms. Consequently the outlining of a rational cultural program should include a knowledge of the time and conditions of differentiation of blossom buds.

Work of this type on other orchard trees, such as the apple, the plum, and the peach, has yielded valuable results in that it has shown the possibility of adjusting methods of culture in such ways as to modify materially the quantity and possibly the quality of the crop of fruit. It is believed that the data presented in this report will afford the basis for similar adjustments in cultural practices with the tung-oil tree.

REVIEW OF LITERATURE

Goff (*2*, *3*, *4*, *5* [3]) working with apples, cherries, cranberries, currants, gooseberries, peaches, and strawberries, determined the time when differentiation of fruit buds occurs and traced the successive stages of development until the unfolding of the blossoms in the spring. He found that the time of differentiation, in the several fruits mentioned as grown in Wisconsin, ranged from June 30 to

[1] Received for publication Nov. 9, 1928, issued June, 1929. This paper is a contribution from the Florida Agricultural Experiment Station. It is adapted from a thesis presented to the faculty of the Michigan State College in partial fulfillment of the requirements for the degree of Master of Science.

[2] The writer wishes to express his thanks and appreciation to L. P. Moore, president of the Alachua Tung Oil Corporation, for financial aid which has made it possible to carry on this work; to Profs. V. R. Gardner, J. W. Crist, and F. C. Bradford, of the Michigan State College, for suggestions in planning and conducting the investigations and for assistance in the preparation of the manuscript; to Prof. W. C. Dutton, of the same institution, for assistance in making the photographs; to Dean Wilmon Newell, of the University of Florida, for assistance in securing aid to carry on this work; to Harold Mowry and B. F. Williamson for suggestions and for the privilege of collecting materials from their orchards, and to Maj. W. L. Floyd, of the University of Florida, for laboratory materials and equipment.

[3] Reference is made by number (italic) to "Literature cited," p. 694.

September 20. In a comparison of apple varieties there was a variation of as much as five weeks in the initial time of fruit-bud differentiation. The time of fruit-bud formation in the cherry and plum was also found to vary by several days in different years. Other investigators have reported subsequently the same conclusions.

In the pecan Shuhart (*8*) and Isbell[4] report that differentiation of the catkin occurs early in the summer previous to blossoming, but the pistillate blossoms are not differentiated until a period in the spring shortly before they blossom.

These citations are by no means a complete résumé of the literature on the subject of fruit-bud differentiation and development in horticultural plants. Furthermore, they do not bear directly on the question as related to the tung-oil tree and are given merely to afford a general perspective of the field. Apparently no data are available on flower-bud differentiation in the tung-oil tree nor its related species.

SOURCE OF MATERIAL

The materials used in this study were collected from two groups of trees at Gainesville, Fla., one on the ground of the experiment station, which will be referred to throughout this publication as Group 1, the other belonging to a private grower which will be referred to as Group 2.

The trees in Group 1 were grown from seed in 1914 and transplanted to the present location in 1917. The soil is very sandy and slopes gently to the south. From 1917 until 1923 the trees were grown under very adverse conditions, being completely surrounded by Carolina cherry-laurel (*Laurocerasus caroliniana*) trees and receiving no fertilizer and practically no cultivation. Since 1923 cultivation has consisted of one plowing during the late fall, followed in the spring by disking. During the remainder of the year the land is hoed frequently to keep down weeds. The trees have received annually an application of 5 pounds of fertilizer (ranging from 4–8–4 to 6–8–5 mixture) per tree, applied about the middle of March. The terminal growth made during 1926 was comparatively short, ranging from 2 to 20 inches, with an average of possibly 7 or 8 inches.

The soil on which the trees in Group 2 are growing is a light sandy loam. The trees are 4 years old and in 1926 each received two applications of Peruvian guano, one in February and the other in July. A cover crop of crotalaria (*Crotalaria striata*) was grown during the summer. This crop was cut and disked under in early December. A second disking was given in March of the following year, and at this time the trees were mulched with mattress-factory refuse, which is composed largely of the outside pulpy portion of Spanish moss (*Tillandsia usneoides* L.) together with numerous small twigs that are removed during the process of ginning. These trees were very vigorous, making terminal growths ranging from 15 inches to 7 feet.

Each sampling consisted of 60 terminal buds taken at random. The first samples were collected on April 19, 1926, from Group 1. Thereafter they were taken each week until September 3, and at 2-week intervals from that date until December. During December, January, and February only three collections were made; these came

[4] ISBELL, C. L. GROWTH STUDIES OF THE PECAN. Ala. Agr. Expt. Sta. Bul. 226, 68 p., illus. 1928.

at intervals of four weeks. Beginning on May 3, 1927, and continuing through June, collections were again made one week apart.

The first collection from Group 2 was made on July 31, 1926; thereafter and until March, 1927, on the same dates as those taken from Group 1.

METHODS

Standard histological methods were used in the preparation of the buds for sectioning and photographing. Killing and fixing were accomplished with picric acid and alcohol solution (1 gm. of picric acid per 100 c. c. of 50 per cent alcohol). Alcohol was employed in the dehydration, xylol in clearing, paraffin for embedding, and Delafield's haematoxylin in staining. A rotary microtome was used in sectioning and sections were cut at 12 microns.

PRESENTATION OF DATA

In this investigation fruit-bud differentiation was considered to have begun when longitudinal sections of the terminal bud showed an elongating growing point with concurrently developing lobes. (Fig. 2, B.) Early progress in the vegetative bud from trees of nonbearing and bearing age is shown in Figure 1. The first evidence of inflorescence differentiation in 1926 appeared in material collected from the trees in Group 1, June 14. Figure 2, A and B, represent two stages of buds collected on that date. The broadening or flattening of the growing point is shown in A, a stage that might be termed the "predifferentiation" stage. This stage is very similar to that observed by Waldo[5] in his study of the strawberry. Figure 2, B, shows the first inflorescence differentiation with potential branches of the flower cluster on either side of the central axis, and Figure 3 shows later developments. Figure 4, A and B, illustrating materials collected July 10, shows an early stage in the development of individual female and male flowers. Figure 4, A, shows an early stage of the pistillate flower with sepals, petals, staminodia, and pistil primordia in the center, while Figure 4, B, shows an early stage in the development of the male flower with sepals, petals, two sets of stamens, and a flattened central portion, indicating that the stamens are not all differentiated. The difference in the length of the central axis of the male and female flower cluster, which continues throughout the development of the bud, is clearly evident at this stage.

By July 17 considerable development had taken place, as is shown by Figure 5, A and B. Further development of the pistillate and staminate flowers is shown in Figures 6 to 8. The crown or central growing axis had divided, showing advancement in the growth of male and female flower parts. In the male flower, Figure 5, B, the sepals are well advanced, the stamens are individually distinct, while the petals, yet in a very embryonic state, appear as pointed projections. In the female flower, Figure 5, A, the pistil primordia, with a small cavity representing early locule development is clearly

[5] WALDO, G. F. FRUIT BUD DIFFERENTIATION AND GROWING HABITS IN CERTAIN TYPES OF STRAWBERRIES. 1926. [Unpublished thesis. Copy on file Mich. State Agr. Col.]

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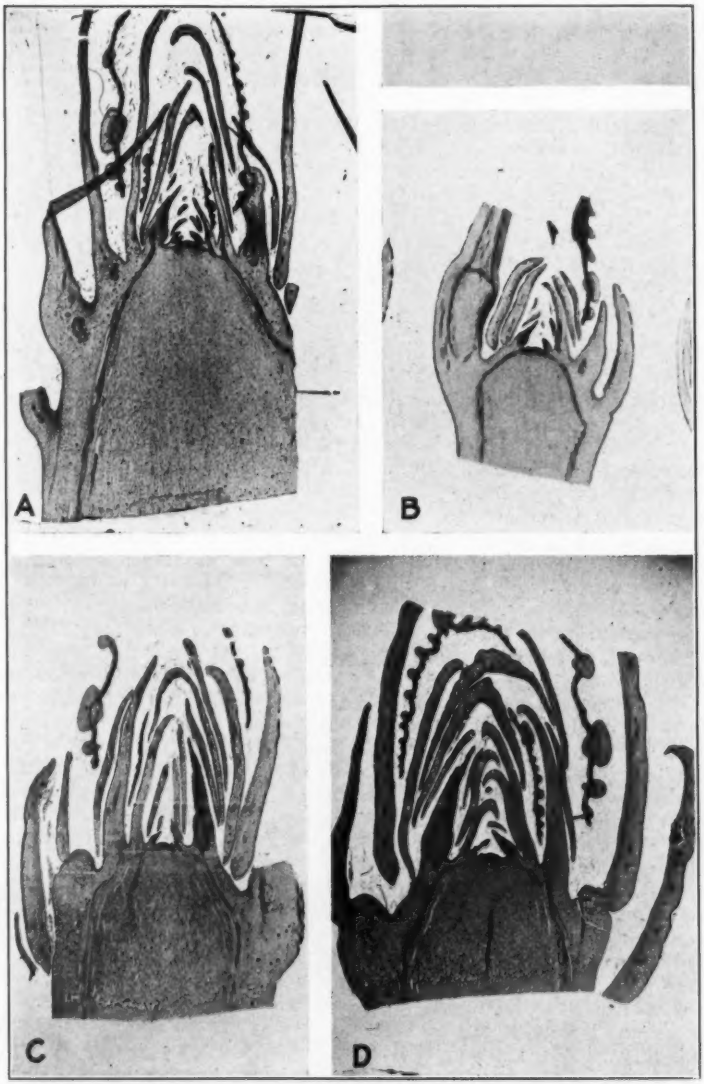

FIGURE 1.—A, Vegetative bud from a nonbearing tree 18 months old; compare with Figure 1, B, C, and D, June 14; B, very early vegetative stage of a bud, from a tree of bearing age, which would apparently have become a fruit bud, April 17; C, progress of development of a bud like that shown in B by May 28; D, development of the vegetative parts of a bud like that shown in B, slight broadening of the growing point is apparent, June 5. × 8

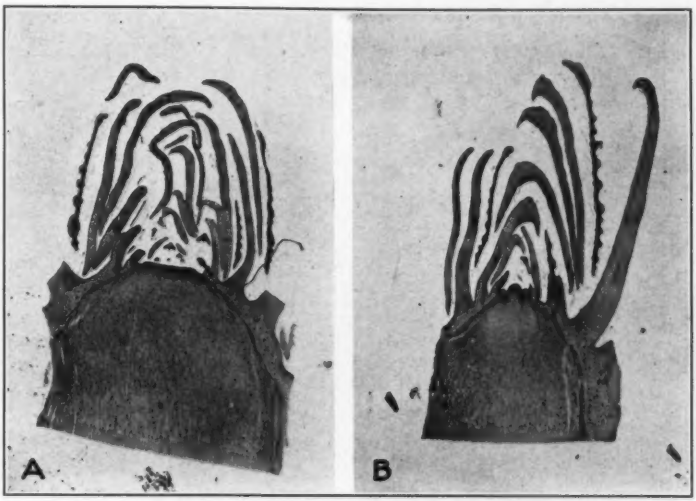

FIGURE 2.—A, Bud from a tree showing a broad, flat growing point at what is considered to be the predifferentiation stage, June 14; B, a very early stage of fruit-bud differentiation, evidenced by the growing point becoming elongated and the lobes advancing on the crown, June 14. × 8

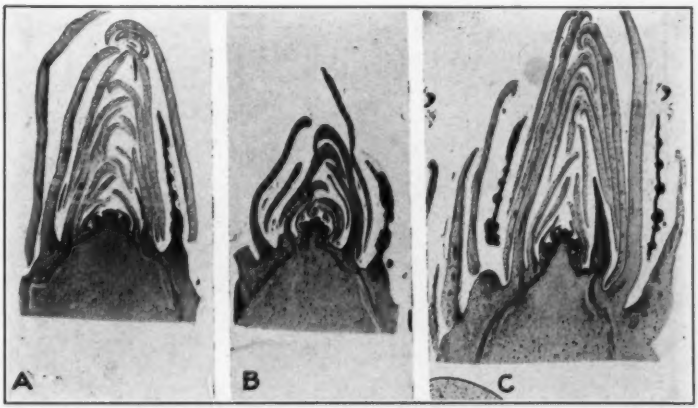

FIGURE 3.—A, Bud as it appeared the second week after differentiation, June 20; B, an early stage in the branching of a flower cluster, June 26; C, the first differentiation of individual flower parts in a bud; rudimentary sepals appear on either side of the tip of the central axis, July 3. × 6.5

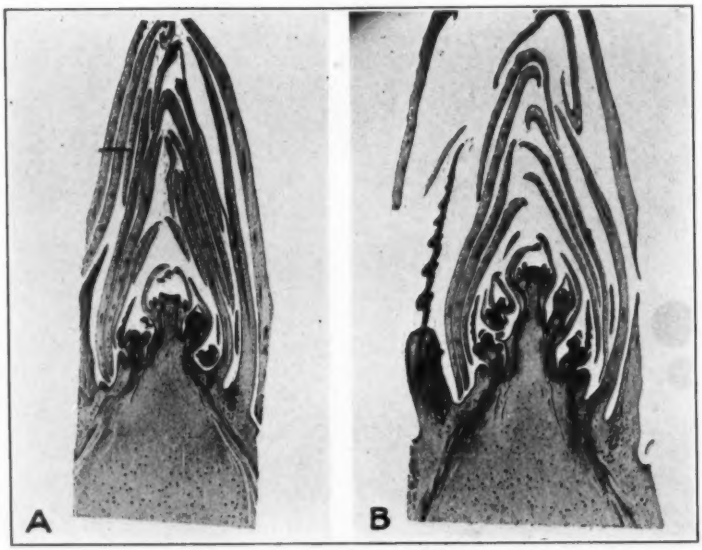

FIGURE 4.—A, An early stage in the development of a pistillate flower; the sepals, petals, staminodia, and pistil primordia are now evident; B, an early stage in the development of a staminate flower; the sepals, petals, and two sets of stamens are evident; not all the stamens are differentiated, as is evidenced by the blunt central portion of the growing point. A comparison between A and B will show the difference in the elongation of the central axis of the male and female flower clusters, July 10. × 8

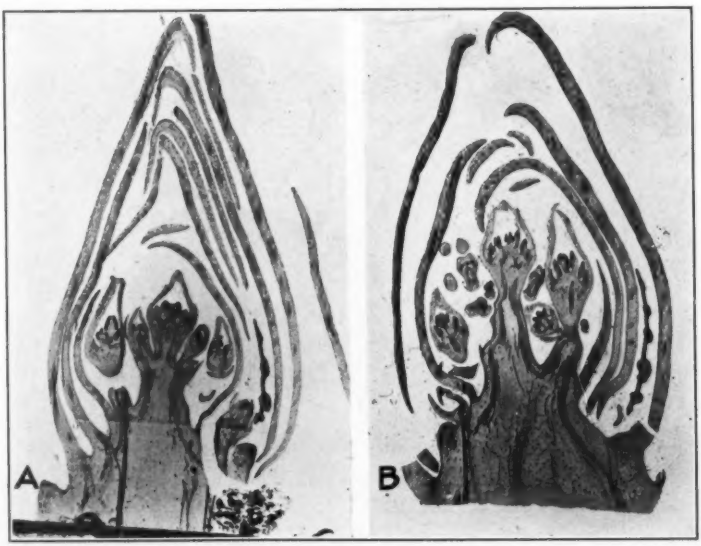

FIGURE 5.—A, Pistillate flower which shows an early stage of locule development near the upper portion of the pistil primordia; B, staminate flower with stamens well advanced; a continued difference in elongation of the central axis of the two flower clusters is evident, July 17. × 8

evident. Between the base of the pistil primordia and the rudimentary petals can be seen staminodia, which rarely develop beyond this stage, as is evident upon comparison with Figure 9, A.

Collections made between July 17 and October 2 (figs. 6 and 7) show in general a progressive increase in size of bud and a general filling out of flower parts already formed. However, little change took place in the individual pistillate flower, other than a slight increase in size of parts already laid down, the pistil remaining in

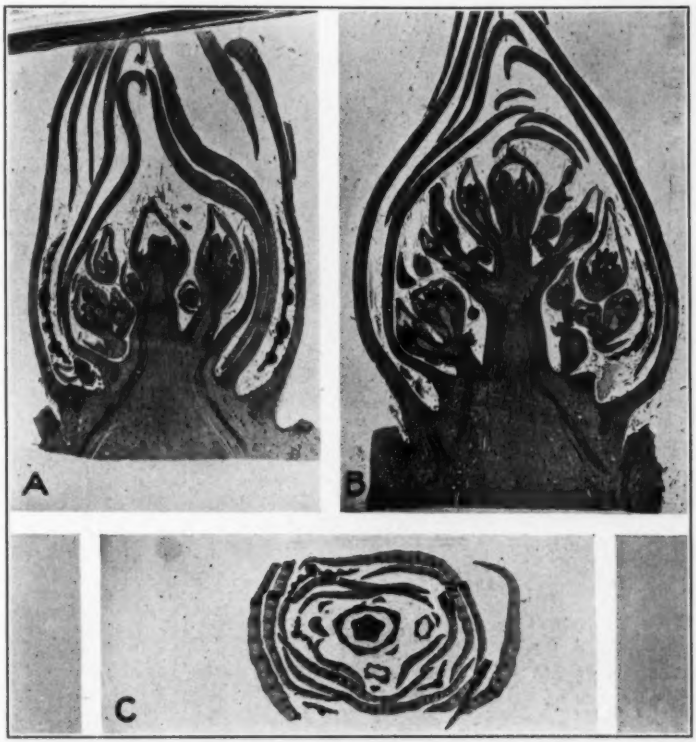

FIGURE 6.—A, Pistillate and staminate flowers developing in the same bud; B, a staminate flower, showing the greater difference in elongation of the central axis of male and female flower clusters; C, a cross section taken through the ovary of a pistillate flower, July 31. × 8.2

an undeveloped condition and the petals only slightly larger than they were on July 16. The pistillate flower is in this condition when development practically ceases, and the flower parts pass through the winter inclosed by bud scales, the outer of which are cemented by an exudation of yellowish-brown resinous material that fills the spaces between the individual flower buds of the cluster.

No great amount of development took place between October 1 and February 5. (Fig. 8.) Shortly before the flower buds begin to unfold growth is resumed and changes take place very rapidly in

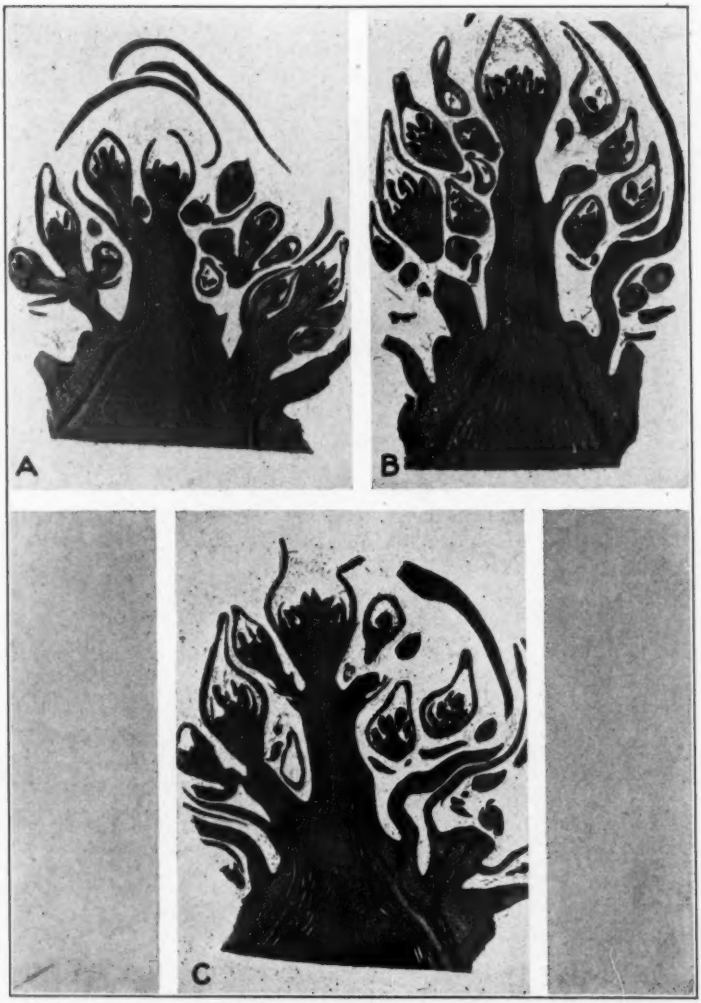

FIGURE 7.—A, A female flower with several branches in the same plane which show male flower development August 14; B, a male flower showing an advanced stage of development and extreme elongation of the central axis, August 28; C, a male flower which has shown little advancement in development after the stage shown in B, October 2. × 8

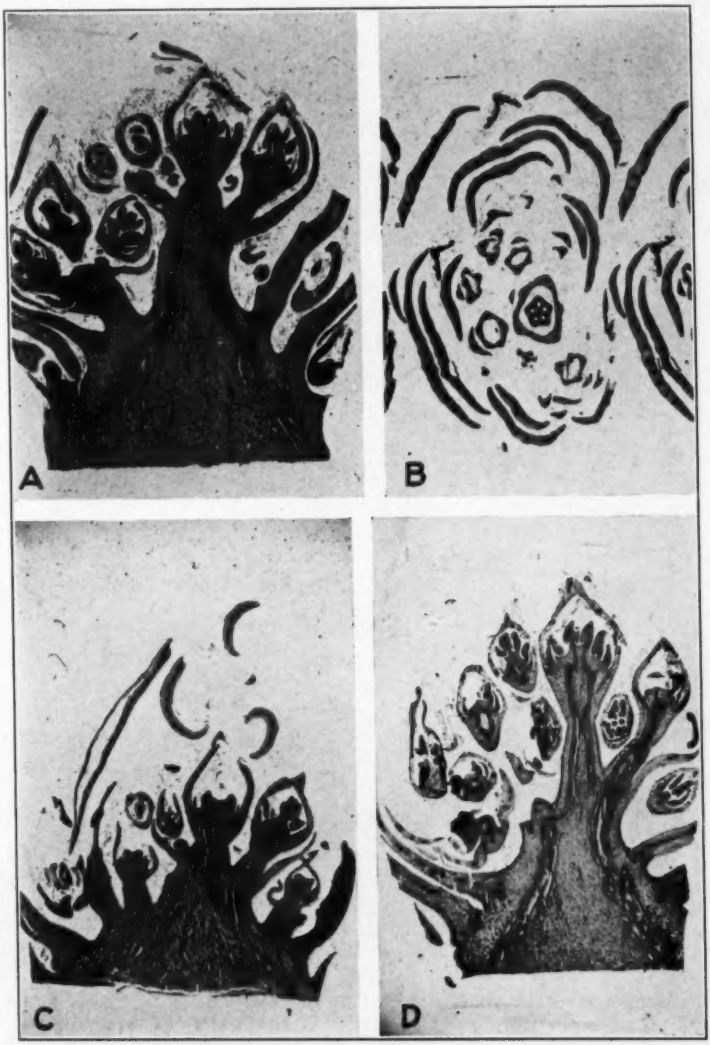

FIGURE 8.—A, A female flower at the stage of development reached before winter, November 13, 1926; B, cross section of the pistil near the base of the ovary, made from the same flower, November 13, 1926; C, pistillate flower which shows little change having taken place during the winter, February 5, 1927; D, a staminate flower which shows little change has taken place during the winter, February 5, 1927. × 8

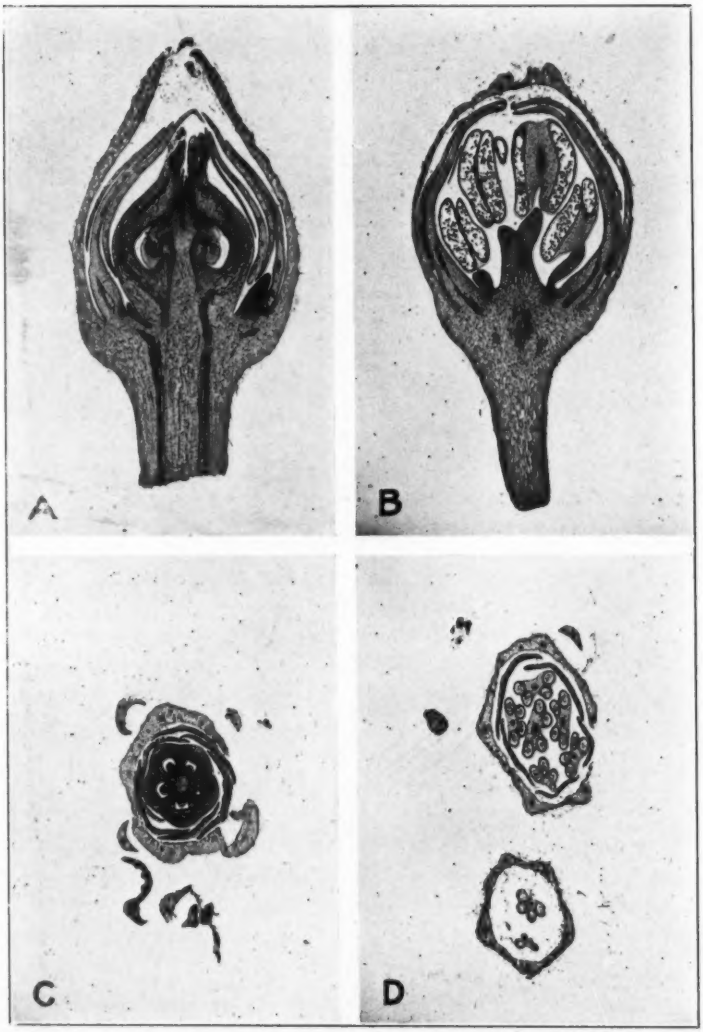

FIGURE 9.—A, A pistillate flower shortly before unfolding and which shows ovule development; B, a staminate flower shortly before unfolding and which shows anthers and pollen grains; C, cross section of the ovary of a flower which shows ovule development; D, cross section of a staminate flower which shows anthers with pollen grains, March 2, 1927. ×8.4

all parts of the individual flower. This is shown by comparing Figure 8, C and D, with Figure 9, A and B, which illustrates the stage of development reached by individual male and female flowers on March 2.

Buds collected in August in Group 2 (fig. 10) did not show differentiation. The first material from trees in this group to show fruit-bud differentiation was collected October 2. At this time, as is evident in Figure 11, A, the central axis is well extended and distinct branching of the flower cluster is evident. The calyx of the central or terminal flower is very conspicuous. On the inner side near the base of the calyx are small rounded protuberances, indicating the initial petals and stamens or staminodia in the case of pistillate flowers. The stage of development at this time (October 20) indicates that differentiation started late in September. The development of the buds for the first eight

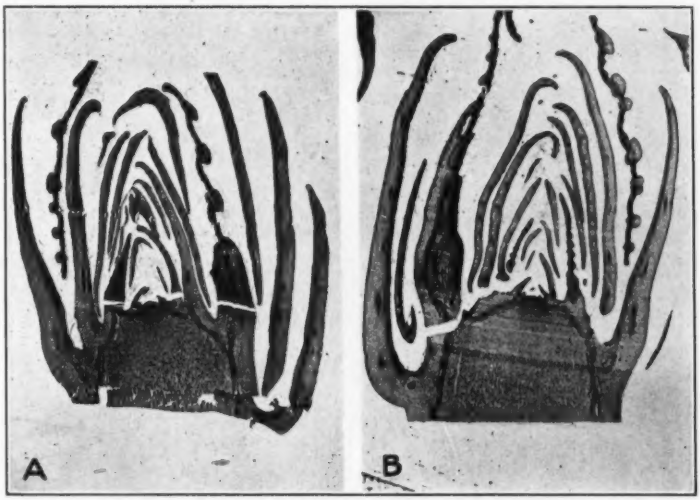

FIGURE 10.—A, Bud from a tree in Group 2 which shows no sign of differentiation, August 14, 1926; B, bud taken from a tree in Group 2 which shows a slight flattening of the growing point which occurs shortly before differentiation takes place, August 28, 1926. × 8

weeks after differentiation was far more rapid than the development of those from Group 1 that were differentiated in mid-June. Although, as shown by Figure 11, C and D, the buds continued to grow and develop until late in December, the rate of growth after November 13 was very much slower than during the earlier stages of development. The changes in development can be seen by comparing Figure 11, B, with Figure 11, C and D. In the flower shown in Figure 12, A, there is no evidence of growth during January.

Some signs of immaturity were noticed in Group 2 when material was collected on December 10 and January 8. On December 10, 1926, the trees retained almost full foliage, but approximately 60 per cent of the leaves (those toward the base of the current wood) showed yellowing preparatory to falling. On January 10, 1927, many leaves which had been killed by frost in late December were still

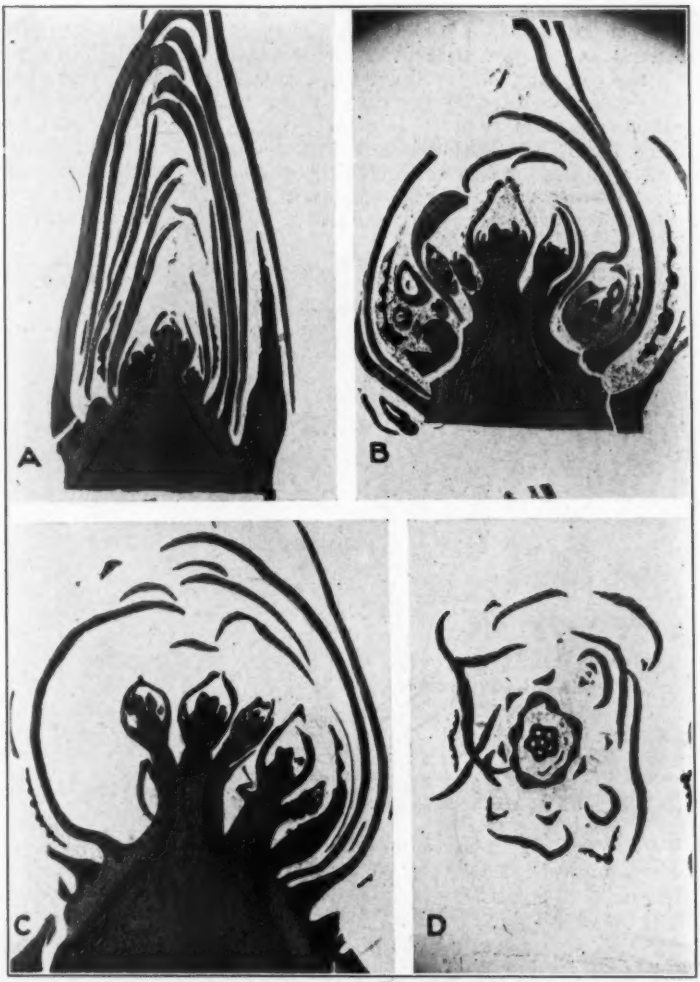

FIGURE 11.—A, Fruit-bud differentiation is shown well advanced and an early stage of individual flowers and flower parts is evident in this bud from Group 2, October 2, 1926; B, pistillate flower showing the rapid development which has taken place since October 2, November 13, 1926; C, pistillate flower showing a further continuation of the development evidenced in B, December 10, 1926; D, cross section of a pistillate flower which shows locules, December 10, 1926. × 8

clinging to the terminal portion of the branches, and wherever this condition exists the buds as well as the terminal portion of the wood were in a slightly soft or wilted condition, indicative of immaturity, though no particular injury from cold could be noted when growth was resumed in the spring.

In sectioning it was found that a much higher percentage of pistillate flowers had been produced on the trees in Group 2 than was found in the material from Group 1. This observation is in line with results reported by Gardner (*1*) concerning sex development in strawberries.

The first indication of fruit-bud differentiation in 1927 was found in material collected from Group 1, May 10. Figure 12, B, shows differentiation well under way on May 17, and Figure 12, C, shows that the stage of development on May 30 was as far advanced as that of material collected July 17 (fig. 5) of the previous year. Furthermore, the rate of development was much more rapid than it was in the first buds to differentiate during 1926. The appearance of pistillate and staminate flowers is illustrated in Figure 13.

The date of differentiation seems to differ considerably from year to year, probably as a result of variations in the time of slowing down of shoot growth. A checking of vegetative growth may be brought about by a number of factors such as a shortage in the amount of available soil moisture, the lack of continuous nutrient supply, method of cultivation, whether sod or clean, the tax of a developing crop of nuts, etc.

Table 1 shows that moisture conditions were strikingly similar in 1926 and 1927 immediately preceding the period of fruit-bud formation and indicates that the difference in time of differentiation in the two seasons was probably due to the drought in April and May in the spring of 1927.

TABLE 1.—*Rainfall in the Gainesville (Fla.) area for the first six months of 1926 and 1927*

Month	Rainfall in—		Month	Rainfall in—	
	1926	1927		1926	1927
	Inches	*Inches*		*Inches*	*Inches*
January	5.59	0.19	April	4.51	0.46
February	1.42	5.78	May	1.60	.46
March	4.93	2.33	June	8.84	10.53

DISCUSSION

Although a large part of the fruit buds differentiate in late June and in July, the data herein presented show that differentation may occur at any time between May 10 and October 1, depending upon weather, cultivation, and varying nutritive conditions. The variation in time of floral differentiation in the two lots of trees is probably due to differences in vegetative vigor and prolongation of vegetative growth. As previously stated, the trees in Group 2 were decidedly vegetative, delaying differentiation until late in the summer.

The observations made during this study indicate that the soil-moisture supply has a rather definite influence upon the time of fruit-

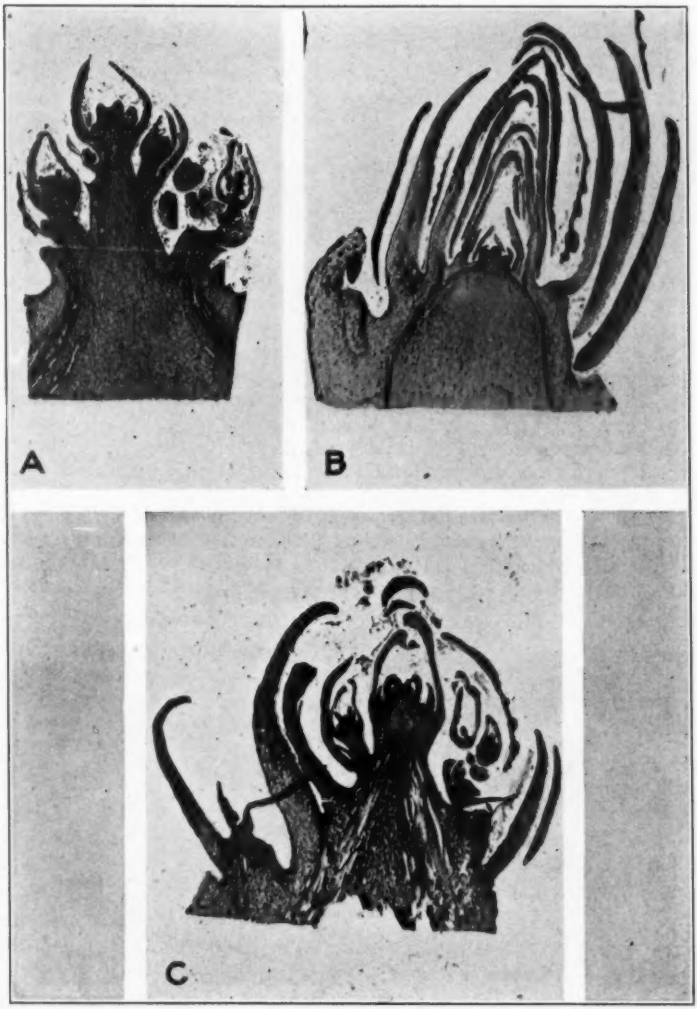

FIGURE 12.—A, Pistillate flower from a tree in Group 2 which shows that little change has taken place in the size of the flower parts during December and January, February 5, 1927; B, bud collected during the 1927 period of flower-bud differentiation which shows differentiation well under way with signs of flower-cluster branches, May 17, 1927; C, pistillate flower collected during the 1927 period of flower-bud differentiation which is as far advanced as those collected July 1, 1926, May 30, 1927. × 8

bud differentiation in the tung-oil tree. While it was not possible to compare irrigated and nonirrigated trees, the results obtained during the two seasons (spring of 1926 and spring of 1927) indicate that an

FIGURE 13.—A, Pistillate flowers of the tung-oil tree; the number of petals varies from 5 to 9, but 8 is by far the most common number in the female flower; B, staminate flowers of the tung-oil tree; the number of petals varies from 5 to 8, but 5 is the most common number in the male flower. ⅔ natural size

increased amount of soil moisture tends to retard fruit-bud differentiation.

The relation between soil moisture supply and time of fruit-bud differentiation observed here in the tung-oil tree is in line with findings of other investigators with various fruit crops. Thus, Kirby (*6*, *p.*

265) in his work with apples grown under varying conditions, says: "The amount of soil moisture is a very important if not the chief external factor in determining the time at which flower buds form." Wiggans (*9*), working with pear and apricot, found that irrigation had a retarding influence on fruit-bud differentiation and development, while Kraus and Kraybill (*7*) in their work on tomatoes say: "Withholding moisture from plants grown under conditions of relative abundance of available nitrogen results in much the same condition on fruitfulness and carbohydrate storage as the limiting of the supply of available nitrogen itself."

Perhaps the most serious objections to prolonged summer growth and late differentiation is the lack of time the tree has properly to develop fruit buds and to mature the terminal portions of the wood sufficiently to pass the winter without frost injury.

The large percentage of female flowers found in material collected from trees in Group 2 compared with the number produced on trees in Group 1 is probably due largely to nutritive conditions. Gardner (*1*) in his work with strawberries found "in most cases * * * femaleness was associated with vigorous growth and good growing conditions generally while maleness or hemaphroditism was associated with less rapid growth and more unfavorable growing conditions." The data that were obtained incident to this study are not conclusive in this respect, but they at least suggest the desirability of keeping the trees in a relatively vigorous condition and thus delaying the period of inflorescence differentiation with the object of promoting the formation of pistillate flowers and preventing irregular bearing due to an alternation of unisexual and bisexual flowering years.

SUMMARY

Fruit-bud differentiation in the tung-oil tree was found to occur at any time between May 10 and October 1, with the majority of the buds differentiating during the latter half of June.

Inflorescence differentiation in 1926 was clearly evident by June 14, and in 1927 by May 10.

The early blossom-bud formation in 1927 indicates that the time of differentiation is hastened materially by a shortage in the amount of available soil moisture.

With the two plots of trees the time of differentiation varied with the vigor of the trees; those that were growing vigorously differentiated floral parts later in the season than those of less vigor.

The indications are that the percentage of pistillate flowers depends upon the vigor of the tree, more female flowers being produced on trees making vigorous growth than on trees showing less vigor.

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EFFECT OF LIME MATERIALS ON THE OUTGO OF SULPHUR FROM HAGERSTOWN SILT LOAM SOIL [1]

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INTRODUCTION

Sulphur is present in soil in different quantities which usually vary with the different geological horizons from which the soil is derived and with the type of agriculture pursued. Sulphur may exist in soil as sulphates, sulphides, and in combination with any organic matter that is present. The amount of sulphur in plants is usually less than 0.5 per cent, and only small quantities of this element are returned to the soil unless an entire crop is turned under.

Sulphur may be added to the soil by snow and rain, the amount brought to the surface by these agencies depending upon the proximity to industrial plants using coal for fuel. In many cases the sulphur added in this manner may replace that which is lost through drainage. It is very necessary that an adequate supply of sulphur be furnished the soil in order that it be available for the important protein molecule of which it is a part. Sulphur is present in cystine and in other amino acids believed to be essential to certain physiological processes in the animal body. Plants may also contain volatile sulphur compounds including glucosides. Sinigrin is an example of such a compound, which, on decomposition in the soil, yields mustard oil, glucose, and potassium sulphate.

Sulphur in the soil may be oxidized or reduced by microorganisms, and the transformation of sulphur is now generally recognized as a biological process. When the sulphur of the soil is oxidized to sulphate it serves as a plant food and may help to maintain a proper physiological balance between the various salts that constitute the food supply of plants. The sulphates commonly occurring in soils are soluble in water and may be lost in the drainage as salts of certain metals.

Modern fertilizer practice calls for the renewal of the plant-food elements taken from the soil by crops or lost by drainage. The application of commercial fertilizers takes care of these losses. However, the concentrated materials used to furnish nitrogen, phosphorus, potassium, and calcium may not contain sulphur, in which case it would be necessary to supply this element unless an adequate supply were brought to the soil by atmospheric agencies or added in the form of organic matter.

[1] Received for publication Jan. 29, 1929; issued June, 1929.

GENERAL PLAN OF THE INVESTIGATION

This investigation had for its object the determination of the loss of sulphur by drainage, its addition by means of certain atmospheric agencies, and the changes brought about by the addition of organic matter and lime from several sources.

The results presented in this paper cover a 6-year experimental period.

REVIEW OF PREVIOUS INVESTIGATIONS

Hart and Peterson (*4*),[2] investigating the sulphur requirements of farm crops, found sulphur to be one of the necessary elements of plant food. Their results show that soil continuously cultivated for 50 to 60 years without the addition of sulphur compounds lost on an average 40 per cent of the sulphur trioxide originally present as compared with that in virgin soils. Where farm manure was applied in liberal amounts the sulphur content was maintained and even increased. The total sulphur trioxide precipitated with the rain amounted in five months to 11.7 pounds per acre. The amount precipitated annually was placed at from 15 to 20 pounds per acre. The losses by drainage based on analyses of the drainage waters at Rothamsted, England, and on an annual drainage of 10 inches, amounted to about 50 pounds per acre annually.

From the data presented these investigators conclude that for increased production of farm crops, systems of fertilization must be adopted that will supply sulphur to the soil in sufficient quantities to compensate for the losses through cropping and drainage. The sources of this element may be farm manures, superphosphates, ammonium sulphate, sulphate potash, gypsum, and elemental sulphur.

Shedd (*7*), who has worked on representative soils from every geological area in Kentucky, found that in nearly all cases constant cultivation without manuring resulted in a loss of sulphur. In many cases this loss was very large when compared with the quantity present in the corresponding virgin soil. There was a variation in the sulphur content of the virgin and cultivated surface and subsoils from the several geological areas and even within the same area. As a general rule, the better agricultural areas showed a higher sulphur content. The virgin and cultivated surface soils as a rule contained more sulphur than their respective subsoils. Shedd concludes that more sulphur is probably lost by drainage than is added in the rainfall and that in order to make up this loss it is necessary to add to the soil some material containing sulphur.

Lyon and Bizzell (*5, p. 72–79*), in their lysimeter investigations at the Cornell University experiment station, made determinations of sulphur regularly in the drainage water and in the crops grown. The annual average sulphur outgo ranged from 31 to 62 pounds per acre. The sulphur removed was considerable, ranging from three to six times that removed by crops, and the ratio varied with the soil treatment. Cropping failed to conserve sulphur as it does with nitrogen. There appeared to be a uniform conversion of sulphur into sulphuric acid in planted and bare soil, and this was found to be sufficient for the needs of the plant. The application of sulphur as potassium

[2] Reference is made by number (italic) to "Literature cited," p. —.

sulphate did not result in an increased crop yield. The application of burnt lime gave an increase in the quantity of sulphur leached. This increase amounted to 9.1 pounds per acre and the biological processes of oxidation appeared to be stimulated by the presence of a sufficient quantity of lime, as is the case with nitrate formation.

Experiments conducted by Hanamann (*3*) with lysimeters show that between April 1 and October 30 the sulphur in the drainage was 44 pounds per acre from a basalt soil and 37 pounds from an alluvial soil. The soils were kept free from vegetation during this period. With a second set of lysimeters maize and red clover were grown. The outgo of sulphur under maize was 29 pounds and under red clover 15 pounds.

MacIntire and Young (*6*) made quarterly analyses of rain water collected at 10 different points in Tennessee and found that on an 8-year average the annual soluble sulphate sulphur precipitation at University farm, on the outskirts of Knoxville, was 51.5 pounds per acre. The 7-year average in the heart of the city of Knoxville was 94.5 pounds, while a 2-year average 7 miles from Knoxville was 18.6 pounds. The authors point out that most of the soluble sulphates found in the rain waters had been dissolved from solid matter and that the volume discharged into the atmosphere is the primary factor governing the concentration of sulphates in the rain water.

Erdman (*2*) collected samples of rain and snow during the years 1920 and 1921 on the agronomy farm of the Iowa Agricultural Experiment Station located 2 miles south of Ames, Iowa, and recorded a rainfall of 38.17 inches. The amount of sulphur added to the soil in the rain and snow amounted to 18.34 pounds. Comparing monthly data for sulphur, he found that the largest amount of sulphur was brought to the soil from March to October when conditions were most favorable for the bacteria which decompose organic matter. Fairly constant amounts of sulphur were found in the rain water for the spring, summer, and fall months, with the exception of July, when only 0.40 pound of sulphur per acre was found.

Eaton and Eaton (*1*) compared the sulphur content of the rain water taken at Chicago, Ill., with that taken at Liberty, Ind., representing a typical agricultural community, and found that the amount of coal consumed in a locality clearly influences the amount of sulphur present in the rain water. The rain falling near Liberty contained for the year less than one-fifth as much sulphur as was found at Chicago. Data are presented to show that the sulphur added to the soil by rain varies directly as the rainfall, but the percentage sulphur content of the rain varied inversely with the amount of rain falling. In conclusion, these investigators find that in agricultural sections where much soft coal is burned the sulphur brought down in the rain may materially supplement the sulphur of the soil and thus delay a sulphur deficiency. That this can only be a delay is indicated by the small amount of sulphur in the soil, the large amount lost in drainage, and the large amount used by crops.

LYSIMETER EQUIPMENT

In 1922 there was installed at the Virginia Agricultural Experiment Station a series of lysimeters for the purpose of studying the income and outgo of the plant-food elements of the soil. These additions and losses are measured by analyzing the rainfall from a rain gauge

and the leachings which pass through known depths of soil in separate soil rims the area of which is known. Tanks of 30-liter capacity are provided to hold the drainage from the rims.

The equipment consists of a concrete pit surrounded by 63 soil rims so constructed that the drainage from each rim can pass through the concrete wall of the pit in block tin tubes into receiving tanks located inside the pit. The lysimeters are located on a terrace, a situation which is advantageous for the disposal of all waste water and allows easy access to the pit. The inside dimensions of the pit are as follows: Length, 43 feet; width, 7 feet; and height, 7 feet.

The walls of the pit are constructed of reinforced concrete 9 inches thick at the base and 6 inches thick at the top. The roof is slightly arched and is constructed of reinforced concrete 5 inches thick. On the end and sides of the inside walls of the pit are heavy oak platforms on which are placed the tanks for holding the leachings. These receiving tanks are made of heavy galvanized iron and have a top through which the leachings pass and which is removable, so that the contents may be accurately weighed on a balance so arranged that it may be moved the entire length of the pit on a track.

The soil rims are constructed of heavy Toncan iron, with bottoms having an opening one-half inch in diameter to which the block tin tubes are soldered. This opening is covered with a fine copper-wire screen and over this, covering the bottom of the rim, are placed three layers of pure quartz, the coarser layer being at the bottom. These layers of quartz act as a filter bed and prevent fine particles of soil from passing into the tanks which hold the leachings. The rims are of three depths, 1 foot, 2 feet, and 4 feet. The area of the rims is one-sixteen thousand five hundreths of an acre.

SOIL

The type of soil used in these experiments is what is known as the Hagerstown silt loam, according to the classification of the Bureau of Chemistry and Soils, United States Department of Agriculture. These soils are considered very productive and are loams and clay loams containing cherty material of different sizes. The soil with which the rims were filled was light gray and ranged in depth from 6 to 10 inches, being underlain by a reddish yellow clay subsoil. The several types of soil classified in the Hagerstown series are derived from the Shenandoah limestone which outcropped in the field from which this particular sample was taken.

EXPERIMENTAL PROCEDURE

In filling the rims with soil an attempt was made to place the soil in approximately the same position as it was found in the field. The soil and subsoil were removed in four layers as follows: 0 to 8 inches: 8 to 20 inches; 20 to 32 inches, and 32 to 44 inches.

In all of the 1-foot rims, soil to a depth of 8 inches was used. The 2-foot rims received weighed amounts of subsoil taken from the 8 to 20-inch layers and also 8 inches of topsoil. To the 4-foot rims equal amounts of subsoil from 8 to 44 inches were placed in the rims and covered with 8 inches of topsoil.

The surface soil was screened through a one-fourth inch seive and thoroughly mixed before it was placed in the rims. The subsoil was

rendered free of all stones and coarse material before it was put in the rims.

The lime materials used in these experiments were marl, limestone dolomite, oyster shells, burnt limestone, burnt dolomite, and synthetic burnt dolomite.

In preparing the synthetic burnt dolomite, pure calcite and magnesite were used. After the proportions had been adjusted the mixture was ignited in a muffle furnace, the resulting product having a value approaching that of a theoretical burnt dolomite. The lime materials were applied at the rate of 2 tons per acre. These applications were made on the basis of calcium carbonate equivalent.

The series of rims to which organic matter was applied received an addition of red-clover hay equivalent to 12 tons per acre.

EXPERIMENTAL WORK

The rims were filled on April 12, 1922, and the soil was allowed to settle until August 29, when the lime materials and organic matter were applied. This time interval is necessary in a soil of this type, for it is liable to fissure badly, which results in an uneven percolation of the rainfall.

Aliquots of each rainfall were taken, and these made up the composites of the annual samples. The aliquots represented one-fiftieth of each rainfall. This amount is amply sufficient for Blacksburg, Va., with a 28-year average annual precipitation of 41.02 inches. The size of the aliquots may be varied to accommodate the number and kind of determinations to be made on the annual samples.

The average annual precipitation for the 6-year period covered in this paper was 38.63 inches. In the 1-foot series 10.18 inches passed through the soil, or a percolation of 26.35 per cent. The 2-foot series gave a percolate of 9.43 inches or 24.41 per cent, and the 4-foot series gave 8.58 inches or 22.21 per cent; therefore, it will be seen, the amount of water percolating through the soil diminished with the depth of soil and subsoil.

About one-third of the annual percolation was obtained during the months of December, January, and February. During this period practically all of the accumulated nitrate nitrogen was washed from the soil. The sulphate sulphur probably followed the same course but to a slightly diminished degree. In 1925 and 1926 droughts occurred, and the small amount of rain that fell during these periods materially affected the outgo of sulphur, especially in the soil rims having a given depth of subsoil.

All of the soil treated, including the controls, maintained an alkaline reaction over the 6-year period. This was also true of the water samples taken from the rain gauge, and indicates that the sulphur brought down by atmospheric agencies was in combination with lime, as the dust particles of the atmosphere in the vicinity of the lysimeters contained alkaline particles, revealing the presence of calcium carbonate. The presence of this basic material was more than sufficient to neutralize any acid produced by the sulphur compounds of the atmosphere in this locality.

The effects produced by the incorporation of clover together with lime in its several forms in Hagerstown silt loam soil are shown in Table 1.

TABLE 1.—*Annual outgo of sulphur over a 6-year period from Hagerstown silt loam soil; lime applications made on a 2-ton basis, $CaCO_3$ equivalent*

[Results expressed in pounds per acre]

WITH ORGANIC MATTER *

Treatment	Rim No.	Outgo of sulphur during—						Total sulphur outgo
		1923	1924	1925	1926	1927	1928	
Rain gauge	49	14.38	17.00	8.20	14.38	27.40	19.86	101.22
1-foot lysimeters:								
Control	45	11.35	21.63	10.76	6.52	15.92	14.02	80.20
Marl	8	15.73	21.53	16.26	7.23	34.90	23.57	119.22
Limestone	9	21.12	15.71	17.89	8.20	34.58	22.10	119.60
Dolomite	10	11.71	17.57	14.04	9.22	34.27	21.01	107.82
Oyster shells	11	18.77	24.47	16.75	9.55	33.98	23.88	126.40
Burnt limestone	12	17.36	19.00	15.40	7.20	34.49	19.05	112.50
Burnt dolomite	13	18.33	28.91	15.54	7.93	33.95	21.02	125.68
Synthetic burnt dolomite	14	22.61	24.23	17.06	8.42	33.94	21.62	127.88
2-foot lysimeters:								
Control	43	16.33	17.81	12.39	3.36	14.43	18.20	82.52
Marl	22	9.91	14.76	16.03	6.40	23.13	21.39	91.62
Limestone	23	9.63	10.03	17.76	6.31	19.53	21.06	84.32
Dolomite	24	12.92	13.99	14.85	7.04	20.91	21.12	90.83
Oyster shells	25	12.98	11.20	15.12	5.21	29.49	20.85	94.85
Burnt limestone	26	13.00	9.17	12.82	7.15	21.81	21.33	85.28
Burnt dolomite	27	10.01	10.17	15.11	8.63	23.10	21.06	88.08
Synthetic burnt dolomite	28	11.98	11.10	14.95	5.34	23.37	21.24	87.98
4-foot lysimeters:								
Control	64	6.26	3.09	2.68	7.83	3.42	1.35	24.63
Marl	57	10.95	2.19	1.74	7.68	4.05	.90	27.51
Limestone	58	13.93	2.12	2.43	6.18	3.85	1.57	30.08
Dolomite	59	24.07	7.77	1.51	6.47	4.33	1.35	45.50
Oyster shells	60	9.74	3.21	1.80	9.96	3.94	1.58	30.23
Burnt limestone	61	27.72	8.38	2.26	9.83	4.22	1.13	53.54
Burnt dolomite	62	19.12	4.04	2.33	5.45	3.93	1.36	36.23
Synthetic burnt dolomite	63	14.45	7.46	2.19	8.62	4.40	1.58	38.70

WITHOUT ORGANIC MATTER

1-foot lysimeters:								
Control	45	11.35	21.63	10.76	6.52	15.92	14.02	80.20
Marl	1	16.94	22.43	14.75	10.85	36.96	21.88	123.81
Limestone	2	11.69	15.86	17.49	10.84	33.24	22.30	111.42
Dolomite	3	11.70	21.22	18.20	10.69	33.60	18.08	113.49
Oyster shells	4	12.79	16.85	16.26	7.53	34.87	21.13	109.43
Burnt limestone	5	12.86	23.62	15.30	8.87	34.49	24.00	119.14
Burnt dolomite	6	13.74	17.94	15.32	11.10	34.41	22.62	115.13
Synthetic burnt dolomite	7	13.24	20.23	16.12	6.72	34.17	22.20	112.68
2-foot lysimeters:								
Control	43	16.33	17.81	12.39	3.36	14.43	18.20	82.52
Limestone	16	8.75	6.65	13.91	5.72	19.87	20.31	75.21
Dolomite	17	5.25	5.77	17.16	7.92	25.60	19.80	81.50
Oyster shells	18	5.89	9.13	18.01	7.60	24.94	21.23	86.80
Burnt limestone	19	6.17	4.49	17.65	7.80	23.72	20.27	80.10
Burnt dolomite	20	7.00	7.14	15.82	8.27	15.89	20.40	74.52
Synthetic burnt dolomite	21	4.54	4.94	12.59	3.71	19.48	20.18	65.44
4-foot lysimeters:								
Control	64	6.26	3.09	2.68	7.83	3.42	1.35	24.63
Marl	15	14.28	1.31	2.11	12.51	3.94	1.12	35.27
Limestone	51	3.23	1.25	.67	4.25	3.94	1.34	14.68
Dolomite	52	4.79	1.00	1.12	1.96	3.36	1.34	13.57
Oyster shells	53	4.69	1.12	1.66	1.15	3.94	1.12	13.68
Burnt limestone	54	16.60	1.49	2.07	5.21	3.65	1.35	30.37
Burnt dolomite	55	25.94	3.41	2.83	5.19	3.94	.90	42.21
Synthetic burnt dolomite	56	15.58	2.38	2.12	5.57	4.78	1.13	31.56
Rainfall (inches)	.....	38.46	41.68	27.82	35.25	44.77	43.82	.....

* Clover hay equivalent to 12 tons per acre.

The rain gauge over a 6-year period gave an annual average outgo of sulphur amounting to 16.87 pounds per acre. The low annual precipitation in 1925 reduced the outgo of sulphur by more than one-half the average, that is, to 8.20 pounds. The rain gauge shows an increase of 21.02 pounds per acre over the control rim of the 1-foot series.

In the 1-foot rims a 12-ton application of clover hay apparently exerted no marked influence on the outgo of sulphur from the soil. In certain instances the clover hay applied gave higher amounts of sulphur in the leachings, but this increase was not consistent throughout the entire series.

An increase in the outgo of sulphur from the series that received an application of clover hay is shown the first two years of the experiment when compared with the series that received none. After the first two years there was very little difference between the two series. During the years 1927 and 1928 the rims which received clover hay closely approached in sulphur leached the series which received an application of lime materials only. The outgo of sulphur in the two groups of experiments with 1-foot rims is practically the same.

The results from the 2-foot rims receiving an application of clover hay show that during the drought years of 1925 and 1926 a diminution in sulphur outgo was apparent. Especially was this true during the second half of this period. This depression of sulphur was just as pronounced in the 2-foot rims which did not receive the clover treatment.

The organic matter applied increased the sulphur leached from the 2-foot rims in certain instances. The range was 1.80 pounds with limestone to 12.33 with oyster shells. The addition of 1 foot of subsoil to the rims showed its effect on the sulphur outgo. The average annual increase of all treated rims over the control in this series was 6.47 pounds of sulphur. The sulphur added by rain and snow was greater in all cases than that removed by the drainage.

The addition of clover hay together with lime materials in 4-foot rims increased the sulphur outgo when compared with that from the control rim. With this depth there was a material increase of sulphur in the leachings the first year of the experiment. In 1924 dolomite, burnt limestone, burnt dolomite, and synthetic burnt dolomite gave slight increases of sulphur leached. The drought in 1925 affected all of the treated rims, the amounts leached being less than from the control. In 1926 the amount of sulphur increased in the leachings, the greatest increase being shown by the rims which received oyster shells, burnt limestone, and synthetic burnt dolomite. The results in 1927 show that the treated rims gave practically the same amount of sulphur leached as was shown by the control. The same conditions prevailed in 1928. In these deeper rims the loss of sulphur was much less than was added in the rainfall.

Lime applications with organic matter in 4-foot rims over a 6-year period gave a greater sulphur outgo than the control. When compared with the control rim, the maximum loss of sulphur was given by the burnt-lime treatment, or 28.91 pounds. The dolomite treatment gave the next greatest loss, or 20.87 pounds.

Among the 1-foot rims which did not receive an application of clover hay the outgo of sulphur was rather uniform for all that had an application of lime materials, but the drought period of 1925–26 reduced the outgo of sulphur during the second half of this period, although the total rainfall was greater. In 1925, the first drought year, all of the leachings were obtained between November and February, the period of least biological activity and of greatest leaching. In 1926, the second half of the drought period, when the

sulphur outgo was extremely low, all of the leachings were obtained after January. The evaporation after January would materially affect the total amount of water passing through the soil.

The minimum effect of lime materials in 1-foot rims was shown in the case of the rim that received 2 tons of oyster shells, after deducting the amount of sulphur that passed through the control rim. This amounted to 29.23 pounds of sulphur per acre. Marl gave the maximum outgo, or 43.61 pounds per acre more than the control. At this depth the biological processes which transform sulphur in the soil appeared to be stimulated by applications of lime.

The leachings from all depths were alkaline during the entire experimental period. This was true of the rain water, control, and treated rims. The amounts of calcium and magnesium in the rain water would maintain this alkaline condition, and it is believed that the sulphur contained in the leachings is combined with the calcium to form calcium sulphate.

In the 2-foot rims receiving lime materials only, the addition of 1 foot of subsoil materially diminished the outgo of sulphur from the soil. The rain gauge gave an increase of 18.70 pounds of sulphur when compared with the control rim. The total amount of sulphur in the rain water for six years was much greater than that of any of the treated rims.

The rim which received an application of synthetic burnt dolomite had an outgo of 35.78 pounds of sulphur less than that of the rain gauge. The maximum outgo of sulphur was shown in the rim which received a treatment of oyster shells. This rim gave an average annual outgo of 14.47 pounds of sulphur per acre.

In all of the rims which received lime materials a much lower outgo of sulphur was shown than from rims receiving similar treatments in the 1-foot series. From these results it is apparent that the addition of subsoil materially depresses the outgo of sulphur with this soil type.

In the treated rims of the 2-foot series without organic matter, oyster shells were the only application which gave a greater outgo of sulphur than the control. During the first two years of the experiment the quantity of sulphur leached from the control rim was almost twice as great as that leached from the treated soils, a fact which indicates that lime applications may depress the outgo of sulphur from the soil.

The 4-foot lysimeters treated with the different forms of lime without the addition of clover hay show striking features in respect to sulphur outgo. Table 1 shows that there is a remarkable difference between the control rim of this series and the controls of the 1-foot and 2-foot series. This control gives 55.57 pounds less sulphur than the 1-foot control and 57.89 pounds less than the 2-foot control, showing that sulphur fixation is brought about by increasing the depth of the subsoil.

The outgo of sulphur from the control rim in this series was 24.63 pounds for the 6-year period, and that found in the rainfall was 101.22 pounds, giving a fixed amount of 76.59 pounds or 75.67 per cent of sulphur fixed. The sulphur fixed by the 1-foot control amounted to 20.77 per cent, and that by the 2-foot control amounted to 18.47 per cent. Not only is the sulphur outgo from the control of the 4-foot series less than from the controls of the other two series but

practically all of the treated rims in the 4-foot series show this fixation of sulphur when compared with the rims having lesser depths of soil and subsoil.

A duplicate series of 2-foot lysimeters was used to study the effect of plant growth on the outgo of sulphur from the soil. These lysimeters were treated with different forms of lime, and in addition one-half of the series received organic matter in the form of clover hay applied at the rate of 12 tons per acre. The clover hay was applied in 1922 when the experiments began, as was done with the uncropped-soil series of experiments shown in Table 1.

The uncropped series of lysimeters was expected to give certain information as to the outgo of sulphur when different forms of lime were added and also when lime was used in combination with clover hay. This series of experiments was outlined for the purpose of studying under similar soil treatments the effects produced by the growing plant on the quantity of sulphur passing out in the drainage.

The 13 rims making up the series were seeded to German millet on June 11, 1924. The millet was harvested on August 30, 1924.

Table 2 gives the weight of the crop and the outgo of sulphur for the year the crop was on the soil and for the year immediately following. The 1925 results are included to show whether or not the millet stubble remaining in the soil affected the outgo of sulphur.

TABLE 2.—*Effect of millet on the annual outgo of sulphur in Hagerstown silt loam soil, lime applications made on a 2-ton basis, $CaCO_3$ equivalent*

Treatment	Rim No.	Weight of crop in 1924	Sulphur leached in 1924	Sulphur leached in 1925
			WITH ORGANIC MATTER [a]	
2-foot lysimeters:		*Grams*	*Pounds per acre*	*Pounds per acre*
Uncropped control	43		17.81	12.39
Marl	36	122.6	11.01	19.38
Limestone	37	124.7	8.23	19.47
Dolomite	38	132.2	9.50	18.16
Oyster shells	39	124.4	12.30	12.11
Burnt limestone	40	144.7	15.42	15.63
Burnt dolomite	41	142.5	18.47	15.87
Synthetic burnt dolomite	42	161.3	20.02	13.79
			WITHOUT ORGANIC MATTER	
2-foot lysimeters:				
Limestone	30	153.2	9.84	13.33
Dolomite	31	123.5	12.81	13.60
Oyster shells	32	112.5	13.40	18.29
Burnt limestone	33	133.1	8.79	16.06
Burnt dolomite	34	108.9	9.28	21.81
Synthetic burnt dolomite	35	112.6	11.17	27.47

[a] Clover hay equivalent to 12 tons per acre.

The plant growth was rather uniform in the entire series of lysimeters, except in those that received limestone and burnt-lime treatments without an application of clover hay. The average sulphur outgo during the year the crop was growing was slightly greater than the amount for the same period leached from the soil that was kept bare of vegetation and under similar treatment. This may be seen by comparing the above results with those for a similar series kept free of plant growth as given in Table 1.

In the series that did not receive an initial application of clover hay, the greatest yield of dry matter was produced with a limestone treatment. The amount of sulphur leached from this rim in 1925 amounted to 13.33 pounds per acre. In rim 16 with a similar treatment, but with no crop, the outgo of sulphur was 13.91 pounds per acre.

The addition of clover hay increased the yield of millet. The increase was shown in every case except that with the limestone treatment. The outgo of sulphur in this series was slightly greater than in the series without the clover-hay treatment. This is shown in the sulphur outgo for 1924. The sulphur leached in 1925 is slightly greater in the series having a clover application. Millet apparently diminishes the sulphur in the leachings to a slight degree, but the year following the crop the outgo appeared to be normal for this particular soil type.

After the millet had been harvested the rims were seeded to red clover August 30, 1924, and this crop was cut on May 22, 1925.

Table 3 shows the effect of clover on the outgo of sulphur in Hagerstown silt loam soil. The yields of clover were increased by the addition of clover hay. The average yield for all of the rims which did not receive the organic matter was 176 gm. Where the organic matter was applied the average yield of all treated rims was 221 gm., or an average increase of 45 gm. The outgo of sulphur was slightly greater for the rim receiving the clover treatment than for the control rim which was uncropped. The quantity of sulphur leached in 1926 was again low and was slightly less than in the series which did not receive clover hay.

TABLE 3.—*Effect of clover on the annual outgo of sulphur in Hagerstown silt loam soil; lime applications made on a 2-ton basis, $CaCO_3$ equivalent*

Treatment	Rim No.	Weight of crop in 1925	Sulphur leached in 1925	Sulphur leached in 1926
		Grams	*Pounds per acre*	*Pounds per acre*
WITH ORGANIC MATTER [a]				
Two-foot lysimeters:				
Uncropped control	43		12.39	3.36
Marl	36	228	19.38	5.20
Limestone	37	218	19.47	4.90
Dolomite	38	219	18.16	5.49
Oyster shells	39	212	12.11	2.65
Burnt limestone	40	230	15.63	4.78
Burnt dolomite	41	219	15.87	5.88
Synthetic burnt dolomite	42	219	13.79	5.66
WITHOUT ORGANIC MATTER				
Two-foot lysimeters:				
Limestone	30	174	13.33	5.57
Dolomite	31	177	13.60	9.27
Oyster shells	32	150	18.29	8.00
Burnt limestone	33	180	16.06	3.91
Burnt dolomite	34	186	21.81	5.28
Synthetic burnt dolomite	35	187	27.47	5.36

[a] Clover hay equivalent to 12 tons per acre.

In the series of rims that did not have an application of clover hay the yields were fairly uniform except for those treated with the caustic forms of lime. Burnt limestone, burnt dolomite, and synthetic burnt

dolomite gave slightly higher yields. In this group of experiments a fairly uniform amount of sulphur was leached from the soil in 1925, but the quantity was greatly diminished the following year. After the crop was harvested 100 gm. of the green clover was returned to each rim. This organic matter, together with the clover stubble, was worked into the soil and followed by a second crop of millet.

In 1925 the largest amount of sulphur which was leached from the bare soil was shown by rim 18, treated with oyster shells. (Table 1.) This amounted to 18.01 pounds. The control of the series showed 12.39 pounds, or a difference of 5.62 pounds. Rim 32, treated with oyster shells, gave in 1925 18.29 pounds of sulphur in the leachings. (Table 3.) This fact shows that practically the same amount of sulphur was leached from a soil kept free of plant growth as from one on which clover was growing.

In 1926 after the soil had received 100 gm. of green clover and the stubble from the crop, the outgo of sulphur from rim 32, treated with oyster shells, was 8.0 pounds per acre (Table 3), while a rim similarly treated but left bare gave 7.60 pounds of sulphur, and the control gave 3.36 pounds (Table 1). The application of clover and the growing of a crop did not materially affect the removal of sulphur from the soil.

The residual effect of clover in combination with lime materials on the outgo of sulphur is given in Table 4.

TABLE 4.—*Residual effect of clover on the annual outgo of sulphur in Hagerstown silt loam soil; lime applications made on a 2-ton basis, $CaCO_3$ equivalent*

Treatment	Rim No.	Weight of crop in 1925	Sulphur leached in—		
			1926	1927	1928
		Grams	*Pounds per acre*	*Pounds per acre*	*Pounds per acre*
WITH ORGANIC MATTER [a]					
2-foot lysimeters:					
Uncropped control	43		3.36	14.43	18.20
Marl	36	228	5.20	23.65	18.27
Limestone	37	218	4.90	22.39	18.47
Dolomite	38	219	5.49	20.28	18.18
Oyster shells	39	212	2.65	20.14	17.70
Burnt limestone	40	230	4.78	18.27	18.53
Burnt dolomite	41	219	5.88	20.56	18.54
Synthetic burnt dolomite	42	219	5.66	17.52	17.74
WITHOUT ORGANIC MATTER					
2-foot lysimeters:					
Limestone	30	174	5.57	31.86	18.66
Dolomite	31	177	9.27	21.06	18.19
Oyster shells	32	150	8.00	16.88	18.53
Burnt limestone	33	180	3.91	19.24	18.24
Burnt dolomite	34	186	5.28	26.62	17.70
Synthetic burnt dolomite	35	187	5.36	25.54	17.73

[a] Clover hay equivalent to 12 tons per acre.

The initial application of clover hay in 1922 to uncropped soils did not materially increase the outgo of sulphur over that from the series on which the clover stubble was turned under. The crop yields in the first group of experiments in Table 4 are much larger than in the second. The sulphur outgo in the two groups shows no marked differences. This holds true for the years 1926, 1927, and 1928, or the years immediately following the harvesting of the clover.

A conservation of sulphur is indicated by the results obtained in 1926, the year following the harvesting of the clover crop. During the years 1927 and 1928 the sulphur content of the leachings again increased. The average annual outgo of sulphur in 1927 for all treatments without organic matter was 23.53 pounds per acre and the average for the same treatments on the rims left bare of plant growth was 21.58 pounds, or a difference of only 1.95 pounds of sulphur leached.

Referring to Table 1, it will be seen that the rain gauge gave 27.40 pounds of sulphur for the year 1927 and the control lost 14.43 pounds. From these figures it can be seen that more sulphur is brought to the soil than is leached from either limed soils or soils receiving both lime materials and organic matter.

The amount of sulphur leached from the treated rims in this series is greater than that leached from the control rim of the series left bare of vegetation.

In 1928 the average amount of sulphur lost from all rims in this series was 18.18 pounds, while the corresponding series of soils left bare of plant growth was 20.37 pounds. This would indicate that clover did not increase the outgo of sulphur from this type of soil.

Table 5 gives the results for the second crop of millet. The millet was sowed on July 5, 1927, and harvested September 26.

TABLE 5.—*Effect of millet, following clover residues, on the annual outgo of sulphur in Hagerstown silt loam soil; lime applications made on a 2-ton basis, $CaCO_3$ equivalent*

Treatment	Rim No.	Weight of crop in 1927	Sulphur leached in 1927	Sulphur leached in 1928
		Grams	*Pounds per acre*	*Pounds per acre*
WITH ORGANIC MATTER [a]				
2-foot lysimeters:				
Uncropped control	43		14.43	18.20
Marl	36	231	23.65	18.27
Limestone	37	320	22.39	18.47
Dolomite	38	281	20.28	18.18
Oyster shells	39	228	20.14	17.70
Burnt limestone	40	277	18.27	18.53
Burnt dolomite	41	283	20.56	18.54
Synthetic burnt dolomite	42	279	17.52	17.74
WITHOUT ORGANIC MATTER				
2-foot lysimeters:				
Limestone	30	182	31.86	18.66
Dolomite	31	170	21.06	18.19
Oyster shells	32	160	16.88	18.53
Burnt limestone	33	186	19.24	18.24
Burnt dolomite	34	170	26.62	17.70
Synthetic burnt dolomite	35	185	25.54	17.73

[a] Clover hay equivalent to 12 tons per acre.

The effect of clover residues on the yield of millet was very pronounced. A marked gain was noted over the first crop, which was harvested in 1924, as can be seen by referring to Table 2.

In 1927 the outgo of sulphur from the soil on which a crop was growing and to which clover was not applied, was greater than in 1928 and averaged 23.53 pounds per acre. Rims receiving the same treatments but left bare of vegetation gave in 1927, taking an average of all treatments, 21.58 pounds of sulphur leached; or the soil growing

the millet lost only 1.95 pounds of sulphur per acre more than did the uncultivated series of rims.

The average quantity of sulphur leached in 1928 was 18.18 pounds. On the series free of plant growth 20.37 pounds of sulphur were lost in 1928. The bare soil lost 2.19 pounds more sulphur than the soil containing the millet stubble.

An initial incorporation of organic matter combined with clover residues and lime materials gave an increased yield of millet. The increases were much greater than those given for the first crop as shown in Table 2 under the same treatment but without clover residues.

The outgo of sulphur in both groups of experiments are rather similar.

These results indicate that the growing of millet and clover tends to diminish the outgo of sulphur, and if there happens to be a diminution following the removal of the crop the sulphur added in the rainfall, together with that contained in plant residues, quickly restores the sulphur to its normal amount for this particular soil type.

DISCUSSION OF RESULTS

In most of the published experimental work involving the losses of sulphur from the soil it appears that serious consideration has never been given to the effects produced by increasing the depth of subsoil, and it is believed that this factor has a very important bearing on the losses of this element by drainage.

Hart and Peterson (*4*) have shown that the total sulphur trioxide precipitated at Madison, Wis., with rain amounted in five months to 11.7 pounds per acre. This amount of sulphur trioxide is equivalent to 4.68 pounds of sulphur. These investigators placed the annual amount of sulphur trioxide precipitated at from 15 to 20 pounds, which would be equivalent to 6 to 8 pounds of sulphur per acre. They contend that "with much less loss by drainage it does not appear that the atmosphere can serve as a complete compensating factor for losses of sulphur trioxide which soils sustain through cropping and drainage."

From an analysis of the leachings from the 4-foot lysimeters used in the experiments reported in this paper, it appears that the rainfall in the vicinity of Blacksburg, Va., would furnish ample sulphur for most crops, and in case of a sulphur deficiency the crop residues usually returned to the soil would be sufficient to take care of the losses.

The average annual precipitation during the six years of this experiment was 38.63 inches. The 1-foot series of lysimeters gave a percolation of 26.35 per cent; the 2-foot series, 24.41 per cent; and the 4-foot series, 22.21 per cent, showing that the addition of subsoil decreased the amount of water passing out as drainage.

About one-third of the annual percolation was obtained during the months of December, January, and February. At the same time practically all of the accumulated nitrate nitrogen was leached from the soil; therefore one would expect the sulphate sulphur to follow the same general course but to a less marked degree owing to the difference in solubility of the two compounds.

A distinct alkaline reaction was maintained in the rain water and the leachings from the control and treated rims. Dust particles in the vicinity of the lysimeters contained calcium carbonate and the ash from the coal used in this area was alkaline. This basic material

was sufficient to consistently maintain an alkaline reaction and overcome any acid effects that the sulphur of the atmosphere might produce.

In the 1-foot series of lysimeters the outgo of sulphur was rather uniform, and the applications of lime in various forms only slightly increased the amount of sulphur in the leachings.

Two drought periods brought out a peculiar feature concerning the outgo of sulphur. These droughts occurred in 1925 and 1926. In the latter year the rainfall was greater, but less sulphur appeared in the drainage water. The larger amount of sulphur leached in 1925 was due to the fact that the drainage was obtained between November and February, the period of greatest leaching in this locality. In 1926, with a greater rainfall and lower sulphur outgo, all of the leachings were obtained after January when the evaporation from the soil would materially affect the amount of water passing through the soil. The period of the year in which the leachings are obtained has a material bearing on the amount of plant food contained in the leachings.

The application of 12 tons of red-clover hay exerted no striking effect on the quantity of sulphur leached from the soil. In certain cases the sulphur increased, but the increase was not consistent throughout the series. Organic matter added to the soil gave slight increases during the first two years after it was applied, but from that time on little increase was noted.

When 1 foot of subsoil was added to 8 inches of soil a diminished outgo of sulphur was noted. This was true when lime materials were used alone and when they were mixed with organic matter. A still greater outgo of sulphur was observed when 3 feet of subsoil were added to the 8 inches of soil. The decrease in sulphur outgo from the deeper rims was consistent throughout the entire 6-year experimental period. It is believed that these clay subsoil additions have a fixing effect on sulphur. This may be a chemical, biological, or physical effect. The sulphur appears to be so held that it is subjected to capillary action within the upper zone of soil during the hotter months of the year. The effect of this action on solutions carrying sulphate sulphur is often noticed in greenhouse and pot experiments, especially with soils high in iron oxide. This action results in the formation of white incrustations at the surface of the soil. This substance may also be observed as irregular white lumps often surrounding a soil particle. On examining these incrustations or lumps it will be observed that they consist largely of calcium sulphate.

It is believed that there is a constant circulation of the soil solution, charged with sulphates, between the upper limit of subsoil and the surface soil, resulting in a deposition of these incrustations. This does not necessarily mean that no sulphur is lost in the drainage, but that the addition of subsoil materially diminishes the sulphur outgo and tends to promote a back-and-forth movement of sulphate sulphur between the subsoil and the surface soil, thereby tending to fix the sulphur.

The growing of crops did not materially diminish the amount of sulphur in the soil. A slight diminution occurred when millet was grown, but the millet stubble left in the soil restored the sulphur the following year. Clover produced no exhaustive effect on the sulphur, and millet, following clover residues, did not reduce the quantity of sulphur.

SUMMARY

The percolation of drainage from Hagerstown silt loam soil varied with the depth of subsoil. About one-third of the annual percolation was obtained during the months of December, January, and February, the period of greatest rainfall.

An alkaline reaction was obtained in the leachings from the rain gauge, control, and treated lysimeters. At no time during the experimental period was the drainage acid or even neutral.

The application of lime materials did not give marked increases in sulphur outgo, but the biological processes which transform sulphur in the soil appeared to be stimulated.

An application of lime materials with which 12 tons of red-clover hay had been mixed did not materially affect the quantity of sulphur that passed out in the leachings.

The growing of millet and clover tended to diminish the outgo of sulphur in the drainage. After the crop was removed the sulphur that was added in the rainfall, together with that contained in the stubble, quickly restored the sulphur to its normal amount for this particular soil type.

Clover residues increased plant growth and, together with the rainfall, added sulphur to the soil in sufficient quantity for a second crop of millet. The bare soil lost 2.19 pounds more sulphur than the soil containing the millet stubble.

Lime materials mixed with clover hay gave much greater yields than lime materials alone. The different forms of lime used gave practically the same crop yields.

The addition of 1 and 3 feet of subsoil diminished the sulphur passing out in the drainage. The total amount of sulphur in the rain water for six years was much greater than in the leachings from any of the rims to which 1 and 3 feet of subsoil were added. This was not the case, however, when 8 inches of soil were added. The addition of organic matter to the lime materials did not alter this depression of sulphur.

The subsoil in these experiments appears to exert a fixing effect in the sulphur brought to the soil in the rain water, for the outgo of sulphur is diminished when the depth of the subsoil is increased.

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